

THE PHARMACOLOGY OF INDOLE COMPOUNDS.

BY

ABDUL HAMEED KHAN,

M.B., B.S., (PUNJAB)

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SECTION I.

HISTORICAL REVIEW.

## Historial Review.

### Introduction.

A number of indole compounds are known to occur in the animal and vegetable kingdoms. Bufotenin (N-dimethyl-5-hydroxytryptamine) was found by Phisalix and Bertrand (1893) in the secretion of the skin glands of the toad. Indole and its 3-methyl derivative, skatole, are found in the large intestine of man (Herter, 1898; Nesbitt, 1899).  $\beta$ -indolyl acetic acid stimulates the growth of plants and is known as a plant hormone (Skoog, 1937; Haagen-Smith, 1939).

These indole compounds have no marked pharmacological activity. Laidlaw (1912) described the actions of tryptamine and it was used as an ecboic under the name of rutamine (Akimoto, 1937). The stimulus to the pharmacological study of indole compounds was prompted when the vasoconstrictor principle, serotonin, present in cattle serum, was identified by the work of Rapport (1949) as 5-hydroxytryptamine.

As 5-hydroxytryptamine is the only indole compound/

compound with marked biological activity so far isolated from mammalian tissues and as it is also the most active indole compound hitherto tested (Erspamer, 1953e), the present study has been undertaken to investigate the actions of this compound on isolated tissues.

It seems possible that a substance which would antagonise the actions of 5-hydroxytryptamine might be valuable in various ways. It might provide evidence for the identification of 5-hydroxytryptamine in tissue extracts. It might help those who are interested in other pharmacologically active substances by suppressing interference due to 5-hydroxytryptamine. It might have direct actions of its own which would provide a clue to the physiological function of the 5-hydroxytryptamine which is found in tissues. It might even have some therapeutic action.

The work presented in this thesis describes mainly, the search for such an antagonist. The early history of various indole compounds will be briefly reviewed.

Indole./

Indole.

Indole and its 3-methyl derivative (skatole) are present in the large intestine as a result of the constant action of putrefactive bacteria (Nesbitt, 1899). Herter (1907) determined the amount as 60 mg of indole and 10 mg of skatole in 100 g of the faeces. It was thought that their absorption from the gut might produce some effects by "auto-intoxication". The earlier work on the pharmacology of these compounds deals mainly with their toxic effects and actions on the central nervous system.

Considerable quantities of indole were administered to dogs, both orally and parenterally, without any toxic effects (Jaffe, 1872; Baumann, 1876; Nencki, 1876; Nesbitt, 1899). Rovighi (1898) found that indole and skatole after intravenous injection in rabbits produced torpor, widespread paresis, feeble heart action and lowered temperature.

A detailed account of the effects of indole in the rabbit, dog, monkey and man was given by Herter (1898). He found that the intravenous injection of indole in both the rabbit and the dog was markedly toxic to the nervous system. He obtained no definite/

definite results when a small, ring-tailed monkey was fed with 0.1 per cent solution of indole for two months. A number of healthy men were able to take, without discomfort, amounts much greater than those ever found in the bowel.

Woolley and Newburg (1911) produced hypertrophy of adrenals and slight interstitial changes in the kidneys of rabbits and white rats by injecting indole in doses greater than that normally found in the intestine of man.

Okhoubo, working with Metchnikoff (1910) studied the effects of these compounds on the blood vessels. He injected indole and skatole repeatedly in rabbits and found arterial changes with indole, but there was no effect with skatole.

The effects of indole were studied by many other workers (Ott and Ulman, 1907; Alvarez, 1924; Bieble, 1929; Houssay, 1936). Skatole was found to be less toxic than indole (Brieger, 1879; Porcher and Hervieux, 1905; Adami, 1914). Indole and skatole inhibit the oxidation of glucose, sodium lactate and sodium pyruvate by the brain (Quastel and Wheatley, 1933). According to these authors, a disturbance of the hepatic detoxicating mechanism might lead to the/

the presence of more than the normal quantities of these compounds in the blood and might produce psychological reactions similar to those met with in anoxaemia or in the early stages of narcosis.

Convulsions have been observed in frogs and rabbits after the injection of indole (Christiani, 1878-1879; Brieger, 1879; Herter and Wakeman, 1899; Danilewsky, 1908; Gautier, 1912, 1914; Bieble, 1929). Bin-ichi-yanai (1935) extensively studied the problem of indole convulsions in frogs and in warm-blooded animals. He came to the conclusion that indole excited the motor parts and depressed the excitability of sensory elements in the spinal cord of these animals. His experiments also showed that  $\alpha$ -methyl and  $\beta$ -methyl indoles paralysed the nervous system of frogs causing complete abolition of spontaneous reflex and respiratory movements. The sensory elements of the cord were especially susceptible to these compounds which thus acted like indole in this respect, but differed from the latter in their effects on the motor elements of the spinal cord, on which the latter exerted an excitant action. The action of indole on the spinal cord of the frog closely resembled that of pyrrol (Tominaga, 1933). Feinberg and/

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and McCullough (1944) applied indole directly to the cerebral cortex of the dog and cat and also injected it intravenously into these animals. They concluded that it acted on many portions of the central nervous system and the characteristic motor seizures observed were of sub-cortical and, in certain cases, of spinal origin.

The actions of indole on blood pressure, respiration, gastro-intestinal tract and the central nervous system of the dog were restudied by Harold and Feinberg (1939, 1942). They injected indole intravenously in a dose of 30 mg/kg and observed a fall in blood pressure, arrest of respiratory movements, diminished intestinal tone, increased secretion of saliva and mucous and spasmodic jerkings in the limbs. All these effects disappeared in fifteen minutes. The administration of atropine stopped the salivary and mucous secretions, but did not change other reactions. A dose of 60 mg/kg of indole killed the animal in five minutes, and at autopsy, a dilated heart, congestion of abdominal vessels and a large amount of mucous in the trachea and bronchi were found. The effects on the central nervous/



nervous system were similar to those observed by Yanai (1935). The above authors also studied the M.L.D. (minimum lethal dose) and M.C.D. (minimum convulsive dose) in albino rats. The M.L.D. was calculated as 37 mg per 100 g weight of rat and M.C.D. as 14 mg per 100 g weight of rat.

Walesch and Rackow (1942) found that indole in a molar concentration of  $10^{-2}$  inhibited serum cholinesterase by 50 per cent and N-methyl indole was at least twice as active.

Guggenheim and Löffler (1916) found that indole produced first a rise and then a fall in the tone of isolated strips of guinea-pig's intestine. Waddell (1927) investigated the actions of indole and skatole on the excised heart of the frog, turtle, cat and rabbit, and noted a diminution in amplitude, rate and output in all these species.

Indole shortens the isolated rectus abdominis of the frog and sensitizes this preparation to the effect of acetylcholine (Torda and Wolff, 1945). Skatole did not modify acetylcholine activity. The effect of potassium was increased by both indole and skatole.

Bunyatyon and Matinyon (1948) described the anti-histamine effects of indole and skatole.

Skatole/



Skatole was the more powerful; 0.35 mg nullified the effect of 1 $\mu$ g histamine.

Izquierdo and Stoppani (1950) confirmed the results of Torda and Wolff (1945) on the rectus abdominis of the frog, but the effect on the smooth muscle was completely different. Both skatole and indole in small concentrations diminished the response of the guinea-pig's ileum to potassium and acetylcholine. The inhibition was reversible.

Izquierdo and Stoppani (1953) confirmed the results of Bunyatyon and Matinyon (1948) and also found that indole and skatole diminished and prolonged the response of the guinea-pig's ileum to acetylcholine. Indole and skatole diminished the tone of the colon of the guinea-pig, rat and mouse. They also diminished the spontaneous contractions of the duodenum of the rabbit and the uterus of the rat, and the response of the latter to oxytocin. These authors suggested, that as indole and skatole were normal constituents of the intestinal fluid (Herter, 1907), they could play a role in intestinal pathology, especially in lesions of the mucosa.

Tryptophan/

Tryptophan.

Tryptophan, the amino acid containing the indole nucleus was discovered by Hopkins and Cole (1902). Various indole derivatives such as indole, skatole, indole propionic acid and indole acetic acid have been linked with the fate of this amino acid (Guggenheim and Löffler, 1916). Tryptophan, like other amino acids is pharmacologically inactive.

Tryptophan-betaine (hypaphorine).

Hypaphorine was isolated by Greshoff (1890) from the seeds of *Erythrina hypaphorus* and was subsequently found in *Erythrina variegata* var. *orientalis* by Maranon and Santos (1932) and in *Erythrina indica* by Rao, Rao and Seshadri (1938). Its possible occurrence in beetroot shoots was suggested by Von Lippmann (1916). It was synthesized by Romburg and Barger (1911).

Hypaphorine produces increased reflex irritability, and later, tetanic convulsions in the frog, but has little action on rats, pigeons, rabbits and guinea-pigs (Plugge, 1893). Chen and Chen (1933a) found it pharmacologically indifferent.

5-indole-acetic acid/

$\beta$ -indole-acetic acid.

$\beta$ -indole-acetic acid stimulates the growth of plants and is known as a plant hormone (hetero-auxin). It is necessary for tuber formation and the curling and bending of plants and is especially present in young growing buds and the leaves (Skoog, 1937; Haagen-Smith, 1939). It is generally held that  $\beta$ -indole-acetic acid in green plants is derived from tryptophan (Jones, Metcalfe and Sexton, 1949). It has been found in the urine of 78 per cent cases of dementia praecox (Ross, 1913). Anderson, Shimi-kin and Leake (1936) observed in mice, the relative toxicity of indole-acetic acid, indolbutyric acid and indole-propionic acid. Indole-acetic acid killed the animals at a dose level of 25 mg/kg, while with indole-propionic acid and indole-butyric acid 100 mg/kg were required. Respiratory embarrassment and failure were the toxic manifestations noted. The toxic effects of indole-acetic acid were also studied by Berthelot et Joseph Dieryck (1939). Like indole and skatole, indole-acetic acid sensitized the rectus abdominis of the frog to the effect of/

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of potassium, while tryptophan was without this action (Torda and Wolff, 1945). Indole-3-acetic acid like skatole but unlike indole, did not modify the action of acetylcholine on the rectus abdominis of the frog.

Indole-ethylamine (tryptamine).

Tryptamine was first obtained by the action of bacteria on tryptophan (Ewins and Laidlaw, 1910). It was later synthesised (Ewins, 1911; Majima and Hoshino, 1925). The pharmacological actions of tryptamine were first described by Laidlaw (1911-1912).

Intravenous injection in the cat or the rabbit, produced a transient stimulant effect causing clonic and tonic convulsions, tremors of the limbs and vasoconstriction.

In the spinal cat, it produced a rise of blood pressure which was less marked in anaesthetised animals. Large doses of nicotine and ergotoxine diminished the blood pressure response to half, while curare had no effect. It stimulated several organs containing plain muscle but the uteri of the rabbit and guinea-pig were relatively insensitive. The action on the cat's uterus was interesting. The virgin/

virgin uterus of the cat was relaxed in vivo but contracted markedly when isolated and suspended in a bath. The pregnant uterus of the cat was stimulated in situ. Laidlaw considered the possibility of two sites of action for tryptamine, on the ganglia and directly on the muscle. Experiments to demonstrate nicotine-like action on the superior cervical ganglia and the splanchnic ganglia failed. He suggested that tryptamine had an action on some peripheral nervous structure, probably a peripheral neurone, which did not survive excision, and concluded that there was a close parallelism between the actions of tryptamine and nicotine on the cat's uterus.

The sphincter as well as the dilator muscle of the iris are stimulated by tryptamine. The sphincter muscle being more powerful ultimately overcomes the dilator muscle and produces slit-like pupil. The effect on the pupil is not observable in cats anaesthetised with chloroform or ether. The effect on the pupil was produced by intravenous injection of the drug in the animal. Hypodermic injection and direct instillation of tryptamine into the eye were without effect.

Tryptamine/

13.

Tryptamine had a mild stimulant effect on the intestine, a weak motor response on the isolated retractor muscle of the penis of the dog and caused marked contraction of the plain muscle of the bladder. The drug diminished the urine flow but this effect soon passed off and was considered to be due to a transient constriction of the arterioles of the kidney.

Guggenheim and Löffler (1916) observed a stimulant effect of tryptamine on guinea-pig's ileum and reported that the drug inhibited the action of histamine on this preparation.

The pharmacological actions of tryptamine were also studied by Shimizu (1916), Matsumura, Nemoto (1924) and Go (1926). Seüchi (1927) studied the action of tryptamine on isolated hearts of toads and rabbits and on vascular preparations of these animals. It was found that tryptamine increased the force of the heart and caused constriction of blood vessels. Small doses of the drug increased the rhythmic activity of the gut but large doses produced inhibition.

Chen and Chen (1933a) reported the same results with tryptamine as Laidlaw (1911). Tryptamine was used as an ecbohic under the name of rutamine, (Akimoto, 1937).

Reid/

Reid (1951) restudied the pharmacology of tryptamine in detail. He confirmed most of the results of Laidlaw (1911-1912). He observed a rise of blood pressure in cats, dogs and rabbits preceded by a fall. There was a rise of pressure in the pulmonary artery or right ventricle which accounted for the initial fall. The pressor response was reduced by yohimbine; the same observation was also reported by Raymond-Hamet (1941). The drug caused bronchoconstriction. It contracted the nictitating membrane, the effect being increased by denervation and depressed by yohimbine. Isolated arterial strips and rat's uterus were also stimulated by tryptamine. Tryptamine (16  $\mu\text{g}/\text{l.}$ ) stimulates the isolated heart of *venus mercenaria* (Twarog and Page, 1953).

Sullivan (1922) succeeded in isolating indole-ethylamine from the urine of pellagra patients. In pellagra, just as in schizophrenia, stuporous and catatonic states were known to occur.

Nieuwenhuyzen (1936) studied the effect of the intravenous injection of tryptamine in cats with the idea that in pellagra and schizophrenia, tryptamine metabolism might have been/



been disturbed. He noticed salivation, narrowing of pupils, negativism and catalepsy in the cat. Tryptamine is oxidised by amine-oxidase (Guggenheim, 1951).

N-methyl tryptamine.

This is identical with dipterin, which occurs in Chenopodiaceen girgensohnia Diptera and Arthropytum leptocladum (Yurashevskii, 1939). It has been synthesized (Manske, 1932; Hoshino and Kobayashi, 1935).

N-methyl tryptamine raises the blood pressure of decerebrate cats, but is less active than tryptamine and N-N-dimethyl tryptamine is still less active. The N-N-N-tri-methyl tryptamine is the most active of these methyl derivatives and has about 1/20 the activity of adrenaline on the blood pressure (Chen and Chen, 1933a). All three methyl derivatives of tryptamine stimulate guinea-pig's uterus and rabbit's intestine, tri-methyl  $\beta$ -indolyethyl iodide is most active on rabbit's intestine. None of them produced any response of the rabbit's pupil.

$\alpha$ -methyl- $\beta$ -indol ethylamine.

This compound was synthesized and its pharmacological/



pharmacological actions extensively studied by Seki (1929), both in vivo and in vitro.

Small and medium doses caused reflex excitability, respiratory stimulation and convulsions in the frog, rat, mouse and rabbit.

The heart, in situ or isolated, was stimulated by small doses but moderate doses diminished the activity of the heart. Peripheral blood vessels were markedly constricted.

The rabbit's uterus was stimulated, the pregnant uterus being more sensitive than the non-pregnant. Ergotamine inhibited the action of the drug on the uterus, but atropine did not affect its response. The drug increased the automatic movements of rabbit's intestine in low concentrations (1 in 100,000), but higher concentrations (1 in 50,000) caused a lowering of intestinal tone and of the frequency of contractions.

Ergotamine inhibited the effect of the drug on the rabbit's intestine, but the action was not as marked as on the uterus. Atropine did not modify the response to the drug. It was suggested by Seki (1929) that the drug had an action on the motor fibres of the sympathetic; in small and moderate doses/

doses it stimulated, but in large doses it paralysed them. He compared its actions with those of tryptamine and concluded that the new-synthetic compound had practical advantages over the older drug, being equally efficient as a uterine stimulant, while weaker in its action on the heart and intestine.

Gramine (3-dimethylamino-methyl indole).

Gramine (Donaxine) was detected by von Euler and Hellström in barley mutants (1932, 1933). It was found in the leaves of *Arnudo donax* L (Muzza and Stolfi, 1931, 1933 and Orechoff and Narkino, 1935). Von Euler and Erdtman (1935) first suggested the identity of donaxine and gramine and represented the alkaloid as 2-methyl amino-3-methyl indole. It was synthesized by Wieland and Hsing (1936) and Kuhn and Stein (1937).

The drug produces a moderate fall of blood pressure in the cat or rabbit, while small doses increase and large doses decrease the blood pressure of the dog (Euler, Erdtman and Hellström, 1936 and Raymond-Hamet, 1937).

Low doses in the mouse produce excitation followed by drowsiness, higher doses cause clonic convulsions./

convulsions. In frogs, low doses cause somnolence, higher doses, paralysis. The drug produces bronchial constriction, increase in tone of the urinary bladder and the intestine of the cat and a fall of temperature in the rat (Supniewski and Serafinowna, 1939). It decreases the amplitude and rate of the isolated heart contractions, causes inhibition, preceded by stimulation of peristaltic movements of the isolated small intestine of the rabbit and contracts the isolated uteri of the rabbit, guinea-pig, rat and Syrian hamster (Powell and Chen, 1945). It reduces, but does not reverse the effects of adrenaline on the blood pressure, intestinal movements and isolated uterus.

Rats are more susceptible to toxic effects of the drug than are mice. The toxicity was determined by intravenous injection.

2-Methyl gramine is similar to gramine for its effects but was 3-4 times more toxic than gramine (Supniewski and Serafin-Gajewska, 1939).

#### Bufotenin.

Bufotenin was found by Phisalix and Bertrand, (1893, 1902), in the secretion of skin glands of the toad (*bufo vulgaris*). It was later isolated from/

from many toads (Handovsky, 1920; Chen, Jensen and Chen, 1931-1932; Jensen and Chen, 1932).

The corresponding quarternary base bufotenidin (Cino-bufotenin) was isolated from the Chinese drug 'Chan Su' and from the skin secretion of many toads (Chen, Jensen and Chen, 1931). 'Chan Su' was prepared from the secretions of the Chinese toad called 'Senso' by the Japanese (Jensen and Chen, 1936). It was in the form of hard, dark-brown smooth round cakes weighing about 56.1 to 82.3 grams. The manner in which these cakes were made was kept secret by the natives. It was interesting that the above workers isolated six compounds from 'Chan Su':-

- 1) cholesterol,
- 2) adrenaline
- 3) cinobufagin,
- 4) cinobufotenin,
- 5) cinobufotoxin,
- 6) suberic acid.

It was used as a haemostatic, cardia-vascular stimulant and analgesic and for a variety of other ailments.

That bufotenin and bufotenidin contained an indole ring was first reported by Wieland, Hesse and Mittasch (1931). The chemical structure of bufotenin was also studied by Jensen and Chen (1932) who expressed the opinion that these substances were derivatives/

derivatives of tryptamine. Their chemical structure was finally established by Wieland, Kanz and Mittasch (1934). Bufotenin was later synthesized (Hoshino and Shimodaira, 1935).

The pharmacological actions of bufotenin were studied by Handovsky (1920) who observed a rise of blood pressure in the rabbit and decerebrate cat, contraction of an arterial ring and myosis in the cat.

Chen, Jensen and Chen (1931-1932, 1932, 1933) and Chen (1934) extensively studied the actions of bufotenin derived from several toads. Several of these bufotenins caused a rise of blood pressure in the pithed cat and had a stimulant action on the isolated intestine of the rabbit and the guinea-pig. They increased the force of the heart and had a slight myotic action when applied locally.

The properties of the pure substance have been studied by Raymond-Hamet (1941, 1942a, 1942b, 1942c) and by Erspamer (1946a). According to Raymond-Hamet (1942a) the rise of blood pressure after the administration of bufotenin was due to stimulation of the adrenal medulla. The uterus of the rat is stimulated by the drug.

Bufotenin is oxidised by amine oxidase (Erspamer, 1946a; Blaschko and Philpot, 1953).

Cino/

Cino-bufotenin stimulates the frog's heart, causes a well-marked rise of blood pressure in the anaesthetised cat, rabbit and dog, powerfully contracts isolated small intestine of the rabbit; has a weak excitant action on isolated uterus of the rabbit and guinea-pig and produces a feeble myotic action (Chen, Jensen, and Chen, 1931; Raymond-Hamet, 1943, Erspamer, 1946a). Its pressor action in the pithed cat was 1/10 of adrenaline (Chen and Jensen, 1931).

Cino-bufotenin, on a weight basis, was approximately one-half as active as 5-hydroxytryptamine but had qualitatively similar reactions except in cats where it was usually pressor when 5-hydroxytryptamine was depressor (Page and McCubbin, 1953a). Yohimbine inhibits the pressor effect of cino-bufotenin in the dog (Raymond-Hamet, 1943).

It stimulates the isolated heart of a mollusc *venus mercenaria* but is 20-100 times less active than 5-hydroxytryptamine (Twarog and Page, 1953).

Cino-bufotenin is also a strong inhibitor of cholinesterase (Sobotka and Antopol, 1937).

Bufothionin./

Bufothionin.

This was another indole compound found in the skin secretions of *bufo gamba*, *bufo arenarum* and *bufo marinus* and also in the Chinese drug 'Chan Su' (Wieland and Vocke, 1930; Wieland, Kanz and Mittasch, 1934 and Jensen, 1935). The chemical structure of bufothionin was established by Wieland and Wieland, (1937).

It is pharmacologically inactive (Erspamer, 1946a, 1952c).

Adrenochrome.

This substance was isolated and identified as quinone resulting from adrenaline oxidation (Green and Richter, 1937). Its solution is very unstable, red in colour and non-fluorescent. It does not possess the classical sympathomimetic actions (Green and Richter, 1937; Philpot, 1940 and Braconier, Lebihan and Beaudet, 1943).

Adrenochrome is known to be an excellent haemostatic so far as capillary haemorrhage is concerned (Derouaux, 1941, 1943; Roskam, Derouaux, Meys and Swalue, 1947). The lack of toxicity and absence of any interfering sympathomimetic action are obvious advantages (Bacq, 1947). It is as good a haemostatic as adrenaline and its action is maximal three minutes/



minutes after injection (Roskam and Derouaux, 1945).

Adrenochrome, like other quinones, catalyses the inactivation of catechol amines and may play a role in hypertension (Oster and Sobotka, 1943).

These investigators, by the administration of adrenochrome and its derivatives, lowered the blood pressure in experimentally induced hypertensive rats but no effect on the blood pressure of normal rats was observed. There were no toxic manifestations.

The work of Martin (1945) and Synder, Leva and Oberst (1947) showed no change in the blood sugar level with adrenochrome.

The actions of adrenochrome on the circulation were also studied by Marquardt and Oettel (1948) and Beau-Villain (1951).

Kuschinsky, Hille and Emmerich (1952a) showed that adrenochrome liberates histamine. When the hind limb of the mouse was perfused with adrenochrome or its semicarbazone, adrenoxyl, there was an increase of oedema as against the control. The outflowing fluid contained histamine or a similar substance. Both adrenochrome and adrenoxyl shortened the bleeding time of the rabbit and this action was prevented by antihistamine. The bleeding time was similarly shortened by histamine and prevented by antihistamines (Kuschinsky, Hille and Emmerich, 1952b).



Adrenochrome in a concentration of 100 mg/l. caused contraction of guinea-pig's and rabbit's ileum. This response was not affected by atropine ( $10^{-9}$ ) but was inhibited by antihistamines ( $10^{-8}$ ). The guts of rats and golden hamsters were less sensitive. Adrenochrome, like histamine, increased the tone of isolated arterial preparations (Kuschinsky, Hille and Emmerich, 1952b).

#### Adrenolutine

The name 'Adrenolutine' was suggested by Lund (1949) for a yellowish-green fluorescent substance formed by the oxidation of adrenaline in alkaline solution. It was proved to be 1-methyl-5, 6-dihydroxy-indoxyl by the above worker.

This substance is the fluorescent product of adrenaline, on the formation of which, the fluorimetric determination of adrenaline is based (Lund, 1949). It is very unstable. The pharmacological actions of this substance are unknown.

#### 5-Hydroxytryptamine (HT)

The interest in the pharmacological study of indole compounds was re-awakened when the vasoconstrictor principle present in beef serum was identified by the work of Rapport (1949) as 5-hydroxytryptamine. Rapport, Green and Page (1948a) called it serotonin/

serotonin and showed that it is present in extracts of serum and that it had a powerful constrictor action on the perfused ear of a rabbit.

The name 'enteramine' was given by Erspamer (1940) to an unidentified smooth muscle stimulating substance present in acetone extracts of gastrointestinal mucosa. He obtained evidence that it was present in acetone and alcohol extracts of the posterior salivary glands of octopoda (Erspamer, 1940, 1948a; Erspamer and Asero, 1953).

The enteramine-like substance appears in the salivary extracts in two forms: A itself active and I itself inactive but easily activated, i.e., transformed into A by simple treatment at pH6-9. Only form A can be directly attacked by enteraminase; form I can be only after activation. The fresh material contains much more substance than stale.

It was also shown to be present in mammalian spleen (Vialli and Erspamer, 1942; Erspamer, 1943a); in the hypobronchial body of muricidia (Erspamer, 1948b) and in amphibian skin (Erspamer and Vialli, 1951, 1952). The presence of enteramine was demonstrated by the above authors by means of colour reactions, pharmacological reactions and by the method of chromatography on paper.

Erspamer/

Erspamer and Faustini (1953) also found enteramine in the serum of vertebrates and in the haemolymph of octopoda.

More recently, enteramine was identified as 5-hydroxytryptamine (Erspamer and Boretti, 1951; Erspamer and Asero, 1952). It seems possible that 5-hydroxytryptamine is also the active substance of the serum vasoconstrictor agent described by Freund (1920) as "Spätgift" and as "Thrombocytin" by Reid (1943). Recently, Quick (1950) suggested a compromise name "Thrombotonin".

It has also been synthesised (Hamlin and Fischer, 1951; Speeter, Heinzelmann and Weisblatt, 1951; Erspamer and Asero, 1952; Asero, Colo, Erspamer and Vercellone, 1952).

Amin, Crawford and Gaddum (1952) presented evidence that 5-hydroxytryptamine is present in certain parts of the brain.

Dalgleish, Toh and Work (1952, 1953) by submitting the acetone extracts of small intestine to counter current fractionation and assaying the fractions on the rat's atropinised colon showed that there were two active principles. One of these active principles was identified as 5-hydroxytryptamine by means/

means of paper chromatography and was distinguished from substance P.

Feldberg and Toh (1953) and Erspamer (1953b,c) studied the distribution of 5-hydroxytryptamine in the wall of the digestive tract.

Twarog and Page (1953) showed that 5-hydroxytryptamine occurs in acetone extracts of dog, cat and rabbit brain in amounts ranging from 0.1 - 0.36  $\mu\text{g/g}$  tissue, and that it is normally excreted in human and dog urine in amounts varying between 0.1 - 1.0  $\mu\text{g/ml}$ . The assay was done on the isolated heart of the mollusc *venus mercenaria* supported by uni-dimensional paper chromatography.

Holgate (1953) found 5-hydroxytryptamine in heparinised blood but not in citrated blood of the rabbit.

Bacq, Fischer and Ghiretti (1952) and Ghiretti (1953) reported the release of 5-hydroxytryptamine by the stimulation of nerves to salivary glands. Humphrey and Jaques (1953) demonstrated the release of a substance with serotonin-like activity in vitro by antigen-antibody reaction. The assay was carried out on rats' colon and atropinised rats' uterus and the/

the identification was based upon specific inhibition by tryptamine and 2-methyl-3-ethyl-5-amino indole.

5-hydroxytryptamine has been identified in wasp venom by paper chromatography and the specific elute found to be pharmacologically identical with this substance (Jaques and Schachter, 1954). Erspamer and Sala (1954) have reported the identification of the stable antidiuretic substance ("Stable ADS") of serum (Ginsburg and Heller, 1951, 1953; Heller, 1952) with 5-hydroxytryptamine.

Taylor, Page and Corcoran (1951) made experiments with cross circulation in dogs and stimulated the vagus centrally. They then detected the release of a substance from the brain, the properties of which appeared to be like those of 5-hydroxytryptamine. This evidence was criticised by Erspamer (1952a) as non-specific.

Recently, Lembeck (1953) has obtained large quantities of 5-hydroxytryptamine in carcinoids, (5 mg per gram of tissue).

Udenfriend, Clark and Titus (1952, 1953) reported the presence of 5-hydroxytryptamine and 5-hydroxytryptophan in the venom of toads (*bufo-marinus*). The above authors showed the presence in/

in animal kidney extracts of a specific de-carboxylase which converts 5-hydroxytryptophan to 5-hydroxytryptamine and produced direct evidence that hydroxylation of the 5 position is a normal pathway in tryptophan metabolism. They also suggested that the presence of methylated derivatives in the toads and other animals may be evidence of a pathway for the metabolism of 5-hydroxytryptamine.

Erspamer and Boretti (1950) identified enteramine by paper chromatography. A fluorescence test for 5-hydroxytryptamine has also been reported (Shepherd, West and Erspamer, 1953; Jepson and Stevens, 1953). Barter and Everson Pearse (1953) detected 5-hydroxytryptamine in mammalian entero-chromaffine cells by histochemical and histophysical methods.

Enteramine has a powerful stimulant action on the isolated atropinised oestrous uterus of rats and mice, the duodenum of the rat, the urinary bladder of the dog, both in vivo and vitro (Erspamer, 1940) and the heart of a mollusc (Erspamer and Ghiretti, 1951).

The isolated heart of a mollusc (*venus mercenaria*) was found to provide a selective and very sensitive method for estimating 5-hydroxytryptamine (Twarog and Page, 1953; Welsh, 1953). 5-hydroxytryptamine increased/



increased the amplitude of the beat in a concentration of  $10^{-9}$  to  $10^{-8}$ , and the effect was immediately reversible. The same authors observed that anterior byssus retractor muscle of *Mytilus edulis*, an edible mussel, was relaxed by 5-hydroxytryptamine.

It was found to have an antidiuretic effect in hydrated rats and dogs (Erspamer and Ottolenghi, 1951; Barac, 1953). Erspamer (1952a) showed that enteramine antidiuresis was due to a reduction of the glomerular filtration rate, as a consequence of a fall in intraglomerular hydrostatic pressure.

This fall in turn was caused by an increase in tone or a spasm of the contractile structures of the afferent vascular bed of the glomerulus. He

suggests that enteramine, the specific storage or secretion product of the entero-chromaffine cell system, may be considered as a true hormone whose task is to control in the rat, and presumably in other vertebrates, the tonus of the afferent vascular bed of the glomerulus and therefore the intra-renal circulatory dynamics (Erspamer and Ottolenghi, 1953). Sala and Castegnaro (1953) carried out numerous researches on the effect of enteramine on the renal function and circulation of the dog. They emphasised that the anti-diuretic action of enteramine, in this experimental animal

was/

was due to stimulation of tubular re-absorptive activity and minimised the role of constriction of the afferent vascular bed of the glomerulus. The mechanism of the anti-diuretic action in physiological doses of enteramine therefore, may be different according to the animal species and perhaps to other conditions.

The pharmacological and physiological actions of enteramine have been extensively studied on various tissues (Erspamer, 1942b, 1943b, 1952b, 1953d, 1953e, and Erspamer and Asero, 1951).

The blood pressure of atropinised rabbits and cats was suppressed by enteramine (Erspamer and Ottolenghi, 1950, 1952). Freyburger, Graham, Rapport, Seay, Govier, Swoap and Vander Brook (1952) reported variable effects on the blood pressure of dogs and cats. Erspamer (1952a) and Page and McCubbin (1953a) explained that enteramine was by no means a pure hypertensive substance in as much as it may possess, according to circumstances, hypertensive, hypotensive and biphasic actions.

Page and McCubbin (1953a) coined a new term "amphibarcic" for the variable pressor effects of 5-hydroxytryptamine.

Reid/



Reid and Rand (1951) showed that 5-hydroxytryptamine on intravenous injection in the cat caused a rise in pressure in the pulmonary artery and a rise of systemic arterial pressure, which may be preceded or followed by a fall. This fall of blood pressure was due to the constriction of the pulmonary vascular bed.

5-hydroxytryptamine constricts the vessels of the hind limb and kidney of the cat, and contracts the isolated carotid artery of the sheep, dog and ox. It liberates adrenaline from the adrenal medulla of the cat and also causes bronchoconstriction and respiratory arrhythmia in the cat (Reid and Rand, 1951). Gaddum, Hebb, Silver and Swan (1953) confirmed the evidence that 5-hydroxytryptamine caused vasoconstriction and bronchoconstriction in cats' lungs perfused with blood.

Douglas and Toh (1952, 1953) found that 5-hydroxytryptamine was a respiratory stimulant in the dog and exerted this effect in several ways which included stimulation of the afferents in the vagus. Schneider and Yonkman (1953) also studied the influence of 5-hydroxytryptamine on vagal afferents impulses in the cat.

The/

The effect of 5-hydroxytryptamine on the circulation and respiration was studied by other workers, (Page, 1952a,c; Reid, 1952; Comroe, 1952; Mott and Paintal, 1953; Heymans and Heuvel-Heymans, 1953). The pharmacological actions of synthetic 5-hydroxytryptamine have been studied by various workers (Reid and Rand, 1951, 1952; Freyburger et al, 1952; Page, 1952a; Erspamer, 1952a, 1953a; Erspamer and Ottolenghi, 1953; Gaddum, 1953a). They have a quantitative resemblance to those of tryptamine (Laidlaw, 1912; Reid, 1951). It potentiates the action of adrenaline on nictitating membrane and blood pressure of the cat (Lecomte, 1953).

Carrel, Lyth, Long and Vanderpoel (1952) studied the haemostatic effect of 5-hydroxytryptamine in rats, guinea-pigs, chickens and rabbits and concluded that it had a marked haemostatic activity but the effect was very short-lived. Page (1952b) also suggested that it had a haemostatic property.

Armstrong, Fry, Keele and Markham (1952) observed a painful reaction after the application of 5-hydroxytryptamine in a concentration of  $10^{-8}$  to the site of a blister induced in the skin by cantharides. Umrath (1953) observed no pain in the cornea when a concentration of  $10^{-8}$  was applied.

Feldberg/

Feldberg and Smith(1953a,b) showed the release of histamine by 5-hydroxytryptamine and tryptamine in the cat, the dog and the rat. The histamine releasing activity of these compounds is somewhat greater than that of propamidine and less than that of 48/80.

Rapport and Virno (1952) found that 5-hydroxytryptamine depressed the rate of oxygen consumption in albino rats when injected intraperitoneally (1 mg/kg). This was not noticed in the rabbit, guinea-pig or dog. Rats were particularly susceptible and showed temporary fore- and hind leg paralysis and narcotic effects. Feldberg and Sherwood (1953, 1954) injected 5-hydroxytryptamine into the ventricle of the brain in the cat and found that 10 µg of 5-hydroxytryptamine had no effect, but 100-200 µg produced licking, profuse salivation, retching, tachypnoea, catatonia and muscular weakness. The cat was not sleepy or drowsy.

Florey and Florey (1953) showed that 5-hydroxytryptamine was a nerve stimulating substance in cephalopodes and crustaceans.

Reid/

Reid and Rand (1951) reported that yohimbine inhibits the pressor action of 5-hydroxytryptamine as well as its stimulant effect on an isolated piece of carotid artery. The pressor action of 5-hydroxytryptamine was also inhibited by 1-hydrazino-phthalazine (c - 5968) (Taylor, Page and Corcoran, 1951), but Erspamer (1952a) reported a non-specific effect with this drug. The modification of the vascular response to 5-hydroxytryptamine by a large number of drugs was extensively studied by Page and McCubbin (1953b).

McCawley, Leveque and Dick (1952) observed sinus tachycardia in anaesthetised dogs after the administration of 5-hydroxytryptamine which was abolished by Apresoline (1-hydrazino-phthalazine c-5968) or veriloid but not with atropine or N-ethyl-N-1-naphthylmethyl-2-bromo-ethylamine (SY - 28).

Woolley and Shaw (1952, 1953c) studied some related anti-5-hydroxytryptamine compounds and found that 2-methyl-3-ethyl-5-amino indole was an antagonist of 5-hydroxytryptamine when tested on isolated ring-shaped pieces of sheeps carotid artery. The corresponding nitro compound when fed to dogs, abolished/

abolished the rise in blood pressure which followed the intravenous injection of 5-hydroxytryptamine but did not affect that due to adrenaline (Woolley and Shaw, 1953a).

Spies and Stone (1952) could not demonstrate any change in the blood pressure of patients with hypertension after 300 mg of 2-methyl-3-ethyl-5-amino indole had been given intravenously over a period of eight hours for 2-3 days or after the oral administration of 22 g for eight days. Page and McCubbin, (1953b) also failed to show any effect with this drug in hypertensive patients. No toxic effects were observed. Iverson and Bull (1953) gave 2:3 dimethyl-5-amino indole to hypertensive subjects with no effect.

Yohimbine and ergotoxine have been reported as naturally occurring anti-metabolites of 5-hydroxytryptamine (Woolley and Shaw, 1953b). Dihydroergotamine and dibenamine have been found powerful antagonists to 5-hydroxytryptamine in its action on rats' uterus and in its anti-diuretic effect (Erspamer, 1952b; Fingl and Gaddum, 1953). Lysergic acid diethylamide (LSD) has been reported to be an extremely potent anti-5-hydroxytryptamine compound on/

on the isolated rat uterus (Gaddum, 1953b).

Antihistamines do not have a marked anti-5-hydroxytryptamine effect (Erspamer, 1952b; Rapport and Koelle, 1953; Herxheimer, 1953a).

Sinha and West (1953) showed on several isolated organs that various local anaesthetics inhibited the response to 5-hydroxytryptamine and their potencies corresponded to their local anaesthetic activity.

Atropine (0.32 mg/kg) gives considerable but incomplete protection against 5-hydroxytryptamine-induced bronchial spasm in guinea-pigs (Herxheimer, 1953a, 1953b). Atropine (10 µg-100 µg per l.) inhibits the effect of 5-hydroxytryptamine on isolated guinea-pig ileum (Rapport and Koelle, 1953; Robertson, 1953 and Rocha e Silva, Valle and Picarelli, 1953). Robertson (1953) and Rocha e Silva et al (1953) also found that hexamethonium had no action on the effect of 5-hydroxytryptamine on guinea-pig's ileum and suggested that the drug acts on the post-ganglionic para-sympathetic fibres.

Evans and Schild (1953) found that 5-hydroxytryptamine produced powerful rhythmic contractions of/  
of/

of the amniotic membrane of 10-12 days incubated chicks. Since the amnion is known to contain plain muscle entirely devoid of ganglion cells and nerve fibres, the action of 5-hydroxytryptamine cannot be due to the release of acetylcholine from nerve endings but is probably a direct action on plain muscle.

Rapport et al. (1948b) reported the presence of an enzyme in extracts of beef and dog lungs which inactivated 5-hydroxytryptamine. Bradley, Butterworth, Reid and Traunter (1950) attempted to characterise the nature of this enzyme and suggested that 5-hydroxytryptamine is oxidised by a mono-amine oxidase in the lung preparation through the same mechanism as that suggested for tryptamine and tyramine. That 5-hydroxytryptamine is rapidly inactivated by amine oxidase has been reported by Freyburger et al. (1952); Blaschko (1952a, 1952b) and Blaschko and Philpot (1953). Blaschko and Hellmann (1953) showed that tissues containing amine oxidase developed a brown colour when incubated with 5-hydroxytryptamine or tryptamine.



SECTION II.

EXPERIMENTAL METHODS.

In the experimental investigations reported hereafter the doses of the following drugs are expressed in terms of the salt:

acetylcholine chloride,  
atropine sulphate,  
mepyramine maleate,  
dibenamine hydrochloride,  
ephedrine hydrochloride,  
benzedrine sulphate,  
ergotamine tartrate,  
ergotoxine ethanesulphonate,  
ergometrine maleate,  
dihydroergotamine methanesulphonate,  
dihydroergokryptine methanesulphonate,  
dihydroergocristine methanesulphonate,  
dihydroergocornine methanesulphonate,  
cinobufotenin flavianate,  
viridobufotenin flavianate,  
marinobufotenin flavianate,  
bufotenin creatinine sulphate,  
hypaphorine hydrochloride,  
mescaline sulphate,  
yohimbine hydrochloride.

The doses of all the other compounds are quoted in terms of the free base, acid or ester.

### Experimental Methods

Isolated oestrous uterus of the rat. This organ was used by Erspamer (1940) as a test object for the detection of enteramine (5-hydroxytryptamine) in tissue extracts. The uterus was suspended in Tyrode solution at 37°C. in 7-8 ml bath. It is difficult to understand why the drug was allowed to act for 150 seconds before washing out, even though a maximum contraction was obtained after about 40 seconds. Erspamer reported frequent spontaneous activity of the preparation.

Erspamer (1942a, 1952b) used ovariectomized rats, and brought them in oestrous by injecting 50-100 µg of oestradiol propionate. A simple method which eliminated the need for ovariectomizing the animals was described by Amin, Crawford and Gaddum (1952). A virgin rat of 160-200 g weight was injected/

injected with 10  $\mu$ g of stilboestrol in 0.1 ml arachis oil per 100 g body weight subcutaneously, and the uterus was used the following day, 20-24 hours after injection of the rat.

The rat was killed by a blow on the head and the uterine horns dissected out. After removing the adherent adipose tissue, a piece of the uterus, about 2 cm long from the middle portion of one horn was tied off at either end and suspended in a 2 ml bath at 30°C. The solution was that formulated by de Jalon and recommended by Gaddum, Peart and Vogt, (1949). It has the following composition (g/l.):

NaCl, 9; KCl, 0.42;  $\text{CaCl}_2$ , 0.06;

$\text{NaHCO}_3$ , 0.5; glucose, 0.5.

Use of this solution with reduced calcium ion and glucose concentration and maintenance of the bath temperature at 30°C. minimized the appearance of spontaneous contractions. It was oxygenated by bubbling through compressed air at a moderate speed. The contractions were recorded with a light frontal writing lever on a smoked drum with a magnification of about 5. The tension on the tissue was of the order of 815 mg. The experiment was started after the/

the uterus relaxed to its fullest extent, which took 30 minutes after it was set up. When a dose of 5-hydroxytryptamine of the order of 10-20 ng was added to the bath a good contraction usually occurred. A delay of 15 to 20 seconds between the addition of the drug and the start of the contraction of the uterus was frequently observed. The contraction was usually completed within 45 seconds after the addition of 5-hydroxytryptamine. and at this time the drug solution was washed out and replaced by fresh de Jalon's solution. Occasionally, the contraction of the uterus required 50 to 60 seconds to reach completion in which case the bath fluid was changed one minute after the addition of the drug. The muscle relaxed to its original length rapidly after the drug had been removed from the bath.

An interval of 4 minutes between doses was usually found suitable. Occasionally, 5 minutes interval between doses was found necessary when the preparation/

41a.

preparation showed tachyphylaxis with the 4 minute interval.

An occasional uterine strip showed marked sensitivity to 5-hydroxytryptamine, an all or none type of response being obtained with doses in the region of 5 ng of 5-hydroxytryptamine. The sensitivity of these preparations was reduced to the normal level by increasing the load on the muscle. The non-oestrous uterus is 10-50 times less sensitive to 5-hydroxytryptamine.

Isolated rat duodenum. Erspamer (1940) described this preparation for testing enteramine-containing tissue extracts. It was suspended in a 2 ml bath containing Tyrode's solution at 37°C. It proved rather insensitive to 5-hydroxytryptamine. A dose of 1 µg of 5-hydroxytryptamine did not give a good response whereas 1 µg of carbachol produced a good contraction. This preparation, therefore, was not considered suitable for testing 5-hydroxytryptamine.

Isolated rat colon. This tissue has been used by Feldberg and Toh (1953) for the assay of enteramine/

enteramine activity. Rat colon (portion adjacent to caecum) was set up in a 2 ml bath in Locke's solution as modified by Gaddum, Peart and Vogt (1949). The temperature of the bath was kept at 22°C. In comparison with the rat's oestrous uterus, the colon preparation proved much less sensitive. A dose of 10 µg 5-hydroxytryptamine in a 2 ml bath produced a small contraction whereas 0.5 µg carbachol gave a marked response. Freyburger et al (1952) also found this preparation less sensitive to 5-hydroxytryptamine.

Isolated guinea-pig's ileum. Guinea-pigs weighing 200-250 g were killed by a blow on the head. The portion of the ileum nearest the caecum was removed and cleaned out with Tyrode's solution introduced into the lumen by means of a pipette. A piece about 2 cm in length was suspended in a 2 ml bath containing Tyrode solution maintained at 37°C through which compressed air was bubbled at a moderate rate. The suspended piece of intestine was threaded with two loops at each end so that the lumen of the intestine remained open. The movements of the gut were recorded with a light frontal-writing lever on a smoked drum with a magnification of 5.

A/



A dose of the order of 50-100 ng of 5-hydroxytryptamine usually gives a satisfactory contraction. Some pieces of ileum removed from a guinea-pig which had been starved a day earlier and set up without any trauma were insensitive to 10-20 ng of 5-hydroxytryptamine. It was also observed that strips of gut which had been kept in Tyrode solution and used a few hours after killing of the animal were less sensitive.

Robertson (1953) reported good contractions of the guinea-pig's ileum with a dose of 2 ng of 5-hydroxytryptamine which were equivalent to those produced by a dose of 4 ng of acetylcholine.

5-hydroxytryptamine was allowed to act for 40 seconds and the time interval between the doses was 3-4 minutes. There was immediate relaxation of the muscle after the drug had been removed from the bath.

On rare occasions, tachyphylaxis to the drug was noted, although Reid and Rand (1952) and Sinha and West (1953) reported this phenomenon more frequently. The preparation gives fairly constant responses, /

responses, but the dose response curve is not as steep as with rat uterus. In general, it proved to be less sensitive than the rat uterus preparation. Various workers (Gaddum, 1953a; Rapport and Koelle, 1953; Rocha e Silva, Valle and Picarelli, 1953) have used this tissue for experiments with 5-hydroxytryptamine.

Isolated guinea-pig's jejunum and duodenum.

These preparations were set up as described for guinea pig's ileum.

The guinea pig's jejunum was used by Reid and Rand (1952) as one of the test objects for studying the effects of 5-hydroxytryptamine. It is ten times less sensitive than guinea-pig's ileum. A dose of 1  $\mu$ g of 5-hydroxytryptamine produced a good response.

The guinea-pig's duodenum was insensitive. A dose of 1  $\mu$ g of 5-hydroxytryptamine gave a small response. The preparation was also not very sensitive to histamine.

The jejunum and duodenum, on account of their relative insensitivity, were not considered suitable for experiments with 5-hydroxytryptamine.

Isolated/

Isolated uterus of the guinea-pig. Both oestrous and non-oestrous uteri were tested. The procedure followed was as described for rat uterus except that the bath contained Dale's solution at 37°C.

The contractions of the uterus (non-oestrous) produced with 5-hydroxytryptamine were very irregular and were characterised by a latency of 45-50 seconds. A dose of 20 µg of 5-hydroxytryptamine in a 2 ml. bath produced a good contraction which was equal to a contraction caused by 0.01 µg of histamine. The relaxation of the uterine muscle was slow after the drug had been removed from the bath. Occasionally, the preparation gave spontaneous muscular contractions after the drug had been washed out.

On the basis of some of the above observations, Reid and Rand (1951) concluded that the preparation was unsuitable for assay of serum vasoconstrictor. Freyburger et al (1952) also found that the responses to 5-hydroxytryptamine were very erratic with this tissue.

Oestrous uterus of guinea-pig. The uterus was brought in oestrous by the injection of stilboestrol as recommended for the rat uterus.

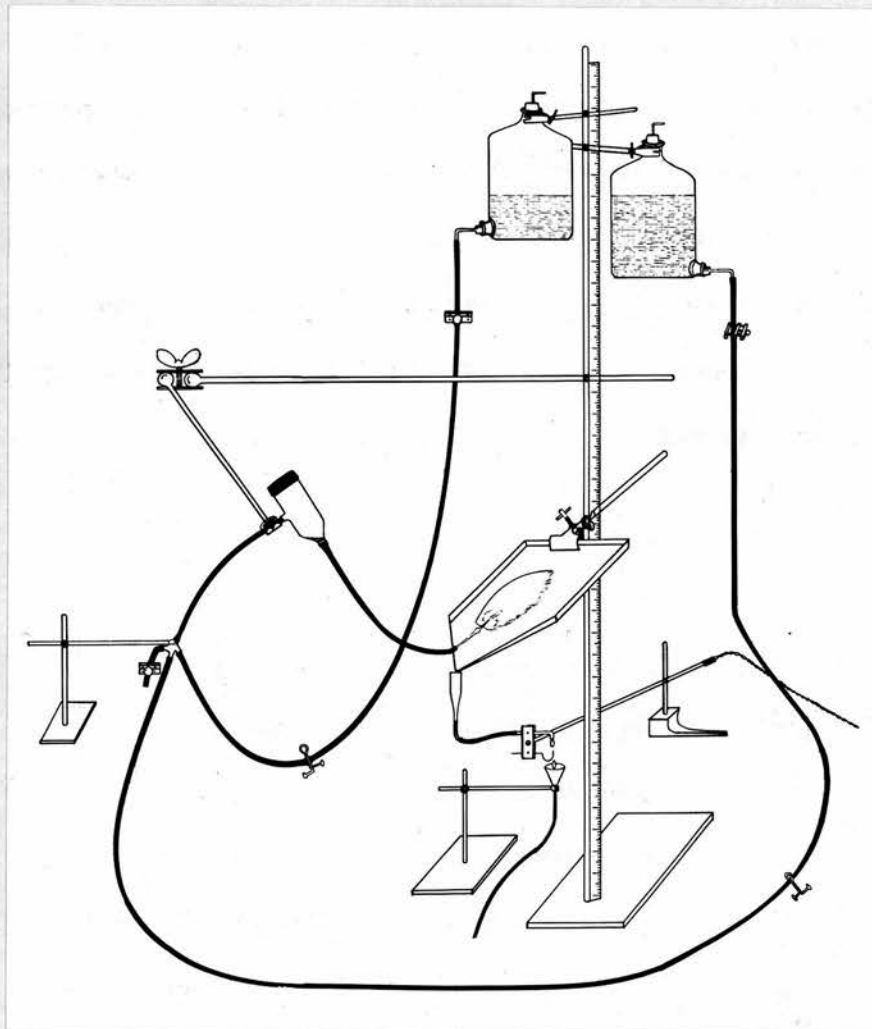
It/

It was found very insensitive to 5-hydroxytryptamine. Even histamine was not active on this preparation.

Perfused rabbit ear. Perfusion of the isolated rabbit's ear was used by Rapport, Green and Page (1948c) for the study of serotonin. Fingl and Gaddum (1953) also used this preparation for testing some of the actions of 5-hydroxytryptamine.

A rabbit was killed by a blow at the back of the neck (not near the base of the ears on the head) and its throat was cut. The ears were severed from the head with a sharp scalpel. The central artery on the dorsal surface of the ear was carefully dissected out and held steady with a piece of thread tied to the end. A small polythene cannula was inserted into the artery and connected to a rubber-capped injection cannula as described by Gaddum and Kwiatkowski (1938). The injection cannula was connected to two perfusion reservoirs, one containing the ordinary perfusion solution and the other containing the antagonist drug. The ear was placed on a draining plate and secured with a pin and the perfusate from the cut surface was led to a small collecting funnel which ended into a horizontal capillary/

Figure 1



Apparatus for perfusion of the  
isolated ear of a rabbit.

capillary tube. The capillary end of the collecting funnel was connected to a drop-timer (Gaddum and Kwiatkowski, 1938). The apparatus is illustrated in Fig. 1).

The ear was perfused at room temperature. Mariotte bottles were avoided as they were liable to introduce irregularities in the flow associated with the escape of each bubble (Gaddum, 1950). The composition of the perfusion fluid was important. It had the following composition as recommended by Page and Green (1948).

NaCl	8.2 g/l.
KCl	0.84 g/l.
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.04 g/l.
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.06 g/l.
$\text{NaHCO}_3$	0.4 g/l.
Glucose	1.0 g/l.

To this is added 10 ml. of phosphate buffer containing 4 parts of 1M  $\text{K}_2\text{HPO}_4$  to 1 part of 1M  $\text{KH}_2\text{PO}_4$ .

When this solution was used, the ear was much more sensitive to vasoconstrictor substances than when Locke's solution was used.

The/



47a.

The ear was perfused under pressure (approximately 35 cm. saline) until blood was no longer evident in the perfusate. The height of the reservoir was then adjusted so that the drop rate of the effluent was 60-70 per minute. This pressure was then kept constant throughout the experiment. A wooden meter rule was fixed to the apparatus to control the pressure.

After the ear had been perfused for an hour, responses to the test agents were elicited. The ear was always more sensitive on the next day after being kept overnight in the refrigerator. It had been observed that the ear could work satisfactorily for three days if kept in proper condition.

The record of the outflow is a series of vertical lines and the height of each line is a measure of the time interval between drops. When a vasoconstrictor substance such as 5-hydroxytryptamine or adrenaline was injected, the heights of the lines/



lines increased owing to lengthening of the intervals between drops of the effluent. Further injections were made when the outflow returned to normal.

A dose of 1-10 ng of 5-hydroxytryptamine usually gave a good response. An occasional preparation showed marked sensitivity, a good response being obtained with doses as low as 0.5 ng of 5-hydroxytryptamine and adrenaline. In comparison with adrenaline, the response to 5-hydroxytryptamine was characterised by a latency of a few seconds and of more prolonged vasoconstriction.

Isolated rabbit duodenum.

Fingl and Gaddum (1953) tested the activity of ergot alkaloids to block the contractions of 5-hydroxytryptamine on the rabbit's duodenum.

A rabbit was killed by heavy blows on the neck and its throat was cut. A piece of duodenum adjacent to the pylorus was removed and the content of/

of the lumen washed out by running in Locke solution from a pipette. Care was taken to avoid excessive trauma. A short segment, about 3 cm long was suspended in a 13 ml bath containing Locke solution maintained at 37°C. and through which air was bubbled at a moderate rate. In most experiments, segments were weighted sufficiently to prevent changes in tone.

After the muscle had been suspended in the bath for one hour, drugs were applied at 5 minutes interval and contractions recorded on a smoked drum. A dose of 10  $\mu$ g of 5-hydroxytryptamine in a 13 ml bath gave a good response. The preparation was not very sensitive and frequently showed marked spontaneous activity.

Rabbit Jejunum. Reid and Rand (1952) reported the stimulant effect of 5-hydroxytryptamine on rabbit's jejunum. The details of setting up the preparation were the same as described for rabbit's duodenum.

It was less sensitive than rabbit's duodenum; a dose of 30  $\mu$ g 5-hydroxytryptamine in a 13 ml bath produced a satisfactory response.

Isolated/

Isolated rabbit uterus. Freyburger et al. (1952) found that 5-hydroxytryptamine was about 1/40 as active as ergonovine maleate on non-pregnant isolated rabbit's uterus.

Non-oestrous and oestrous uteri were tested.

Non-oestrous uterus. A small strip of one horn was suspended in a 2 ml. bath containing Locke's solution maintained at 37°C.

A dose of 10 µg 5-hydroxytryptamine in the 2 ml. bath gave a good contraction, but the response to subsequent doses was reduced. Adrenaline in a concentration of 0.1 µg/ml. produced a satisfactory response.

Oestrous uterus. The uterus was brought in oestrous by the injection of 100 mg/kg of stilboestrol. The injections of stilboestrol were given subcutaneously for two consecutive days and the animal was killed on the third day. A piece of a uterine horn was suspended in a 2 ml. bath containing Locke's solution maintained at 37°C.

The preparation gave marked spontaneous activity. The temperature was lowered to 30°C. but the spontaneous activity persisted.

De Jalon's solution, as recommended for rat's uterus, was substituted in place of Locke's solution. It/

It reduced the spontaneous activity. A dose of 1  $\mu$ g of adrenaline in the 2 ml bath gave a good contraction, but 5-hydroxytryptamine in a dose of 50  $\mu$ g was without much effect.

Isolated rabbit's uterus, was thus not a suitable tissue for experiments with 5-hydroxytryptamine.

#### Summary

5-hydroxytryptamine was found to cause contraction of the uterus, duodenum and colon of rats, duodenum and jejunum of rabbits, and the uterus, duodenum, jejunum and ileum of guinea-pigs and vasoconstriction in the perfused rabbit's ear.

The most sensitive of these tissues were the rat's uterus (Erspamer, 1952a); the guinea-pig's ileum (Gaddum, 1953a; Robertson, 1953; Rocha e Silva, Valle and Picarelli, 1953) and the rabbit's ear (Rapport, Green and Page, 1948c), all of which have been used before for experiments with 5-hydroxytryptamine. These three sensitive tissues have been used in most of the experiments recorded in the present study.



**SECTION III.**

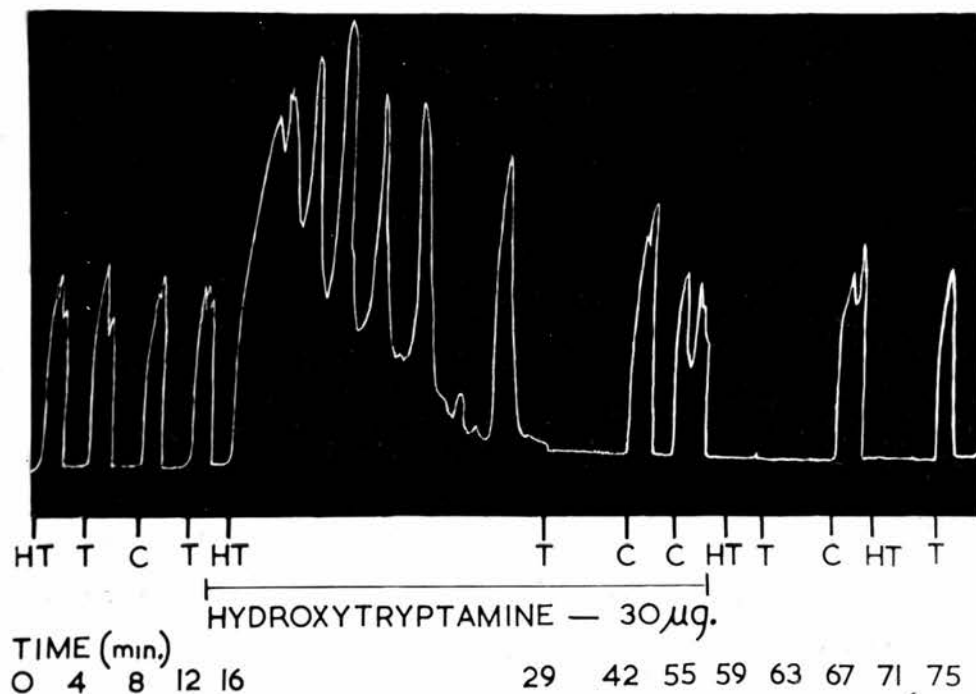
**DRUGS WHICH ANTAGONISE 5-HYDROXYTRYPTAMINE.**

Drugs which antagonise 5-hydroxytryptamine.

There is some evidence that some of the actions of 5-hydroxytryptamine (HT) and tryptamine are due to combination with special receptors, which are not identical with the receptors for histamine, adrenaline or acetylcholine and which have been called tryptamine receptors (Gaddum, 1953a). These other drugs all have effects like those of HT on some tissues but not on all tissues. Histamine and adrenaline do not stimulate rat's uterus; adrenaline does not stimulate guinea-pig's ileum and acetylcholine causes vasodilatation rather than vasoconstriction in the freshly prepared rabbit's ear (Burn and Robinson, 1951).

In every case, a test of specificity was carried out by comparing the effect of HT with that of a suitable dose of some other drug before and after the addition of the antagonist. When the effects of both drugs were depressed the antagonism was considered unspecific and unimportant. Such effects may be caused by depression or poisoning of the/

Figure 2



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine 150 ng

T - tryptamine 15000 ng

C - carbachol 1500 ng

16-56 min. a high concentration of HT (15000 µg/l.) abolished the responses to tryptamine and HT but not that to carbachol. On removing HT from the bath, tryptamine caused a response after 15 min.

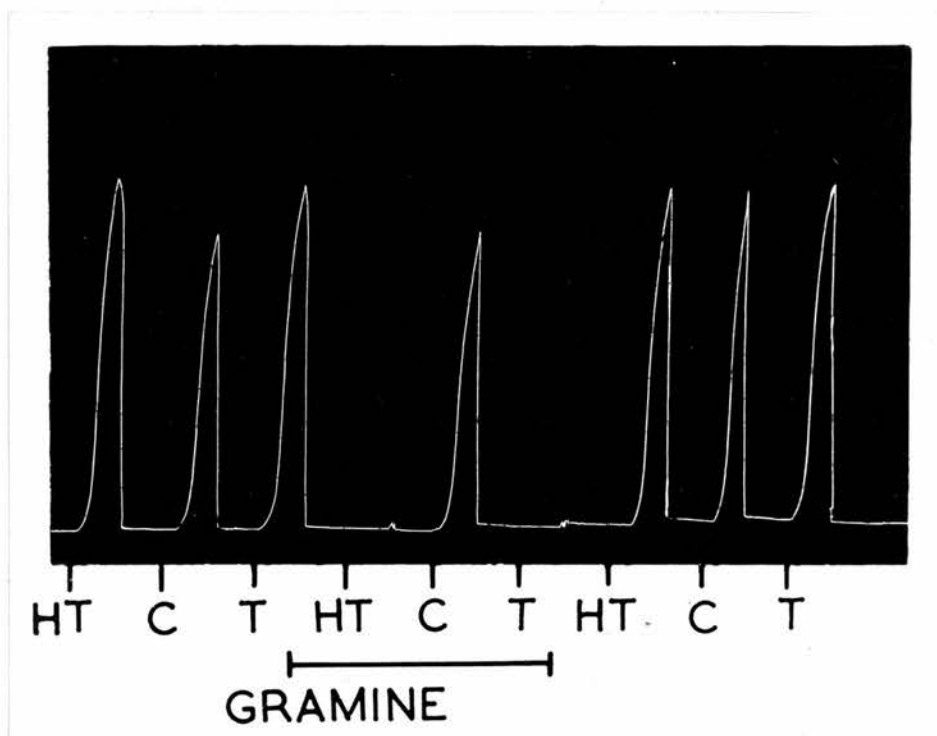


the contractile mechanism. The statement that an antagonism was specific means that the effect of 5-hydroxytryptamine was depressed while the effect of the control drug was not. This test does not prove that 5-hydroxytryptamine and its antagonist are competing for the same receptor, but does exclude some cases in which they are not. The usual control drugs were acetylcholine for rat's uterus, histamine for guinea-pig's ileum and adrenaline for rabbit's ear.

Tachyphylaxis. Guinea-pig's ileum can be specifically desensitized to 5-hydroxytryptamine by exposure to high concentrations of this drug or tryptamine (Gaddum, 1953a; Rocha e Silva et al. 1953).

Similar results may sometimes be obtained with the rat's uterus. In one experiment, (Fig. 2), 5-hydroxytryptamine (75  $\mu\text{g}/\text{l.}$ ) and tryptamine (7500  $\mu\text{g}/\text{l.}$ ) caused effects similar to those of carbachol (750  $\mu\text{g}/\text{l.}$ ). A high concentration of 5-hydroxytryptamine (15,000  $\mu\text{g}/\text{l.}$ ) was added to the bath for 43 minutes. This caused strong contractions which disappeared after 13 minutes. The muscle now gave no response to 5-hydroxytryptamine or tryptamine, but the effect of carbachol was unchanged. This desensitization/

Figure 3



Rat's uterus - 2 ml. bath - interval 3 min.

HT - 5-hydroxytryptamine 20 ng

C - carbachol 100 ng

T - tryptamine 4000 ng

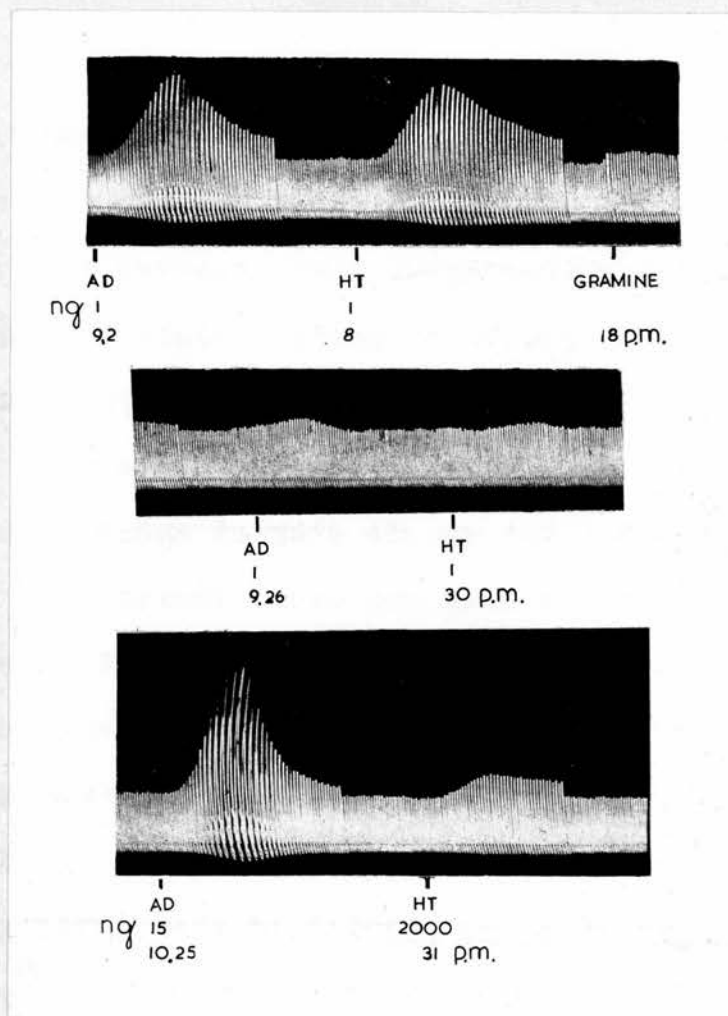
Gramine (10 mg/l.) specifically inhibited tryptamine receptors.

desensitization persisted for 15 minutes after the high concentration of 5-hydroxytryptamine had been removed; the muscle then again gave a response to tryptamine. In some experiments, the effect was obscured by the persistence of muscular contraction in the presence of 5-hydroxytryptamine, or tryptamine. The inhibitory effects of high concentrations of 5-hydroxytryptamine or tryptamine were much less clearly shown on the rat's uterus than on the guinea-pig's ileum. It would not be easy to do similar experiments with the rabbit's ear because large doses of 5-hydroxytryptamine stop the flow completely.

Specific antagonisms are commonly due to the competitive action of drugs similar in structure to the active drug and tests have therefore been made with a number of compounds which, like 5-hydroxytryptamine, contain an indole nucleus. Most of these were specially prepared by Messrs. Glaxo Labs. and the results will be described in subsequent study, (Section IV).

Gramine (3-dimethyl amino methyl-indole) acted as the prototype for a number of active synthetic anti-5-hydroxytryptamine compounds and its effect on the/

Figure 4.



Rabbit's ear perfused. Height of record shows interval between drops.

AD - adrenaline

HT - 5-hydroxytryptamine

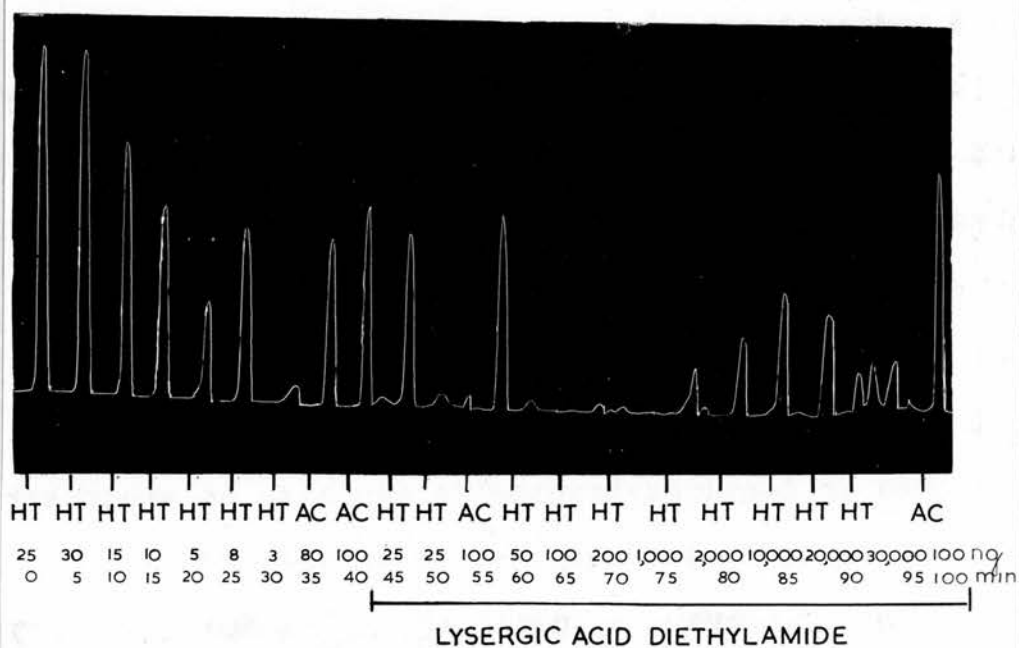
From 9.18 gramine (10 mg/l.) was continuously present in the perfusion fluid. Both drugs antagonised, but HT more than adrenaline.

the rat's uterus is shown in Fig.3. It abolished the response to 5-hydroxytryptamine without affecting the response to carbachol. The effect immediately recovered after the drug was washed out. Erspamer (1954) has observed the same effect. A concentration of less than 10 mg/l. of gramine did not inhibit the 5-hydroxytryptamine response to a marked extent. It is liable to produce muscular contractions especially if kept for more than 10 minutes in the bath. Higher concentrations of the drug caused contractions and the uterus started spontaneous activity after the drug had been removed from the bath. In a concentration of 10 mg/l. it decreased the sensitivity of the rabbit's ear to 5-hydroxytryptamine by more than two-thousandfold and that to adrenaline by about fifteenfold (Fig. 4). It caused slight vasoconstriction of the rabbit's ear. On guinea-pig's ileum, its action against 5-hydroxytryptamine was feeble and non-specific. Histamine was also markedly inhibited.

Ergot alkaloids. Tryptamine forms part of the molecule of lysergic acid, certain derivatives of which are very active antagonists of 5-hydroxytryptamine on the rat's uterus and the rabbit's ear.

The/

Figure 5



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine

AC - acetylcholine

Lysergic acid diethyl-  
amide (100  $\mu$ g/l.) specifi-  
cally inhibited the response  
to HT.



The antagonism of ergotoxine and tryptamine was described by Laidlaw and that of ergotamine and  $\alpha$ -methyl tryptamine by Seki (1929).

Heymans, Bouckaert and Moraes (1932) found that ergotamine antagonised the vasoconstrictor action of defibrinated blood which was presumably due mainly to 5-hydroxytryptamine. Gaddum, Peart and Vogt (1949) obtained similar results with dihydroergotamine and made the additional observation that suitable concentrations of this drug suppressed the response of the rabbit's ear to cat's plasma without altering the response to adrenaline. This fact seemed surprising at the time, but is now explained since the response to 5-hydroxytryptamine can also be suppressed without altering the response to adrenaline.

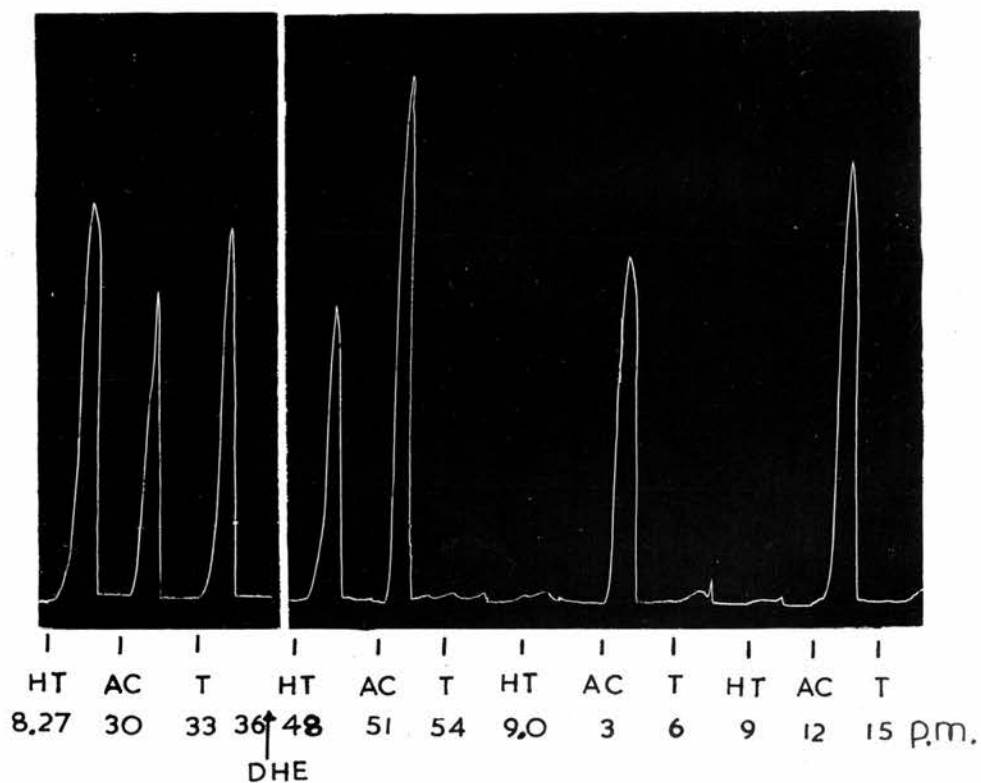
The antagonism of ergot alkaloids and 5-hydroxytryptamine has been studied more recently by Erspamer (1952b), Shaw and Woolley (1953), Fingl and Gaddum (1953) and Page and McCubbin (1953b).

Lysergic acid diethylamide (LSD) has been reported by Gaddum (1953b) as the most active and specific of these derivatives.

Figure 5 shows the result of an experiment on the/



Figure 6



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine 14 ng

AC - acetylcholine 80 ng

T - tryptamine 1600 ng

DHE - dihydroergotamine (50  $\mu$ g/l)  
 for 10 min and washed out.  
 The response to HT was  
 abolished, but that to  
 acetylcholine potentiated.

the rat's uterus in which lysergic acid diethylamide (100  $\mu\text{g}/\text{l.}$ ) inhibited the effect of 5-hydroxytryptamine completely without affecting the response to acetylcholine. When lysergic acid diethylamide was left in the bath, its activity continued to increase and a dose of 30,000 ng of 5-hydroxytryptamine did not give much effect after about an hour.

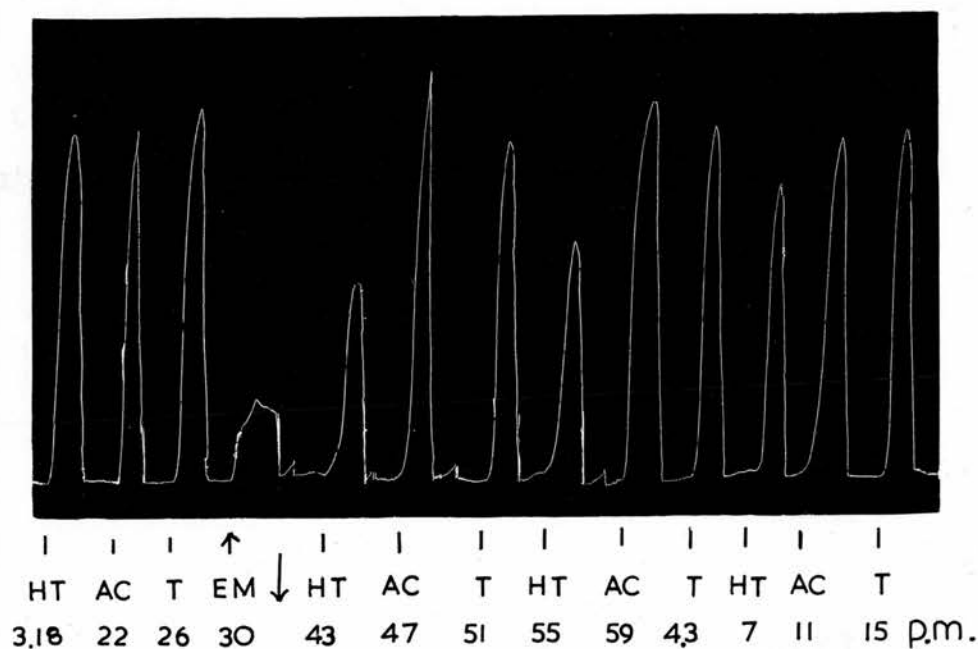
Lysergic acid diethylamide in a concentration of 10  $\mu\text{g}/\text{l.}$  or even less, completely inhibits the effect of 5-hydroxytryptamine on rat's uterus.

Dihydroergotamine in a similar experiment specifically inhibited the response to 5-hydroxytryptamine (Fig. 6 ) and the effect continued to increase with time.

Lysergic acid diethylamide and dihydroergotamine in higher concentrations tend to cause contractions of the uterus especially if kept for longer periods in the bath.

A series of derivatives of lysergic acid have been tested for their effects on the rat's uterus as antagonists of 5-hydroxytryptamine. Their relative activities have been determined quantitatively and will be described under section IV. They/

Figure 7



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine 20 ng

AC - acetylcholine 200 ng

T - tryptamine 3000 ng

EM - ergometrine (500 µg/l.) for 10 min and washed out.

Note the partial inhibition of the response to HT and the potentiation of the response to acetylcholine. The responses to both drugs subsequently show a gradual return to original levels.

They are placed in the following descending order of potencies: lysergic acid diethylamide,

    dihydroergotamine,

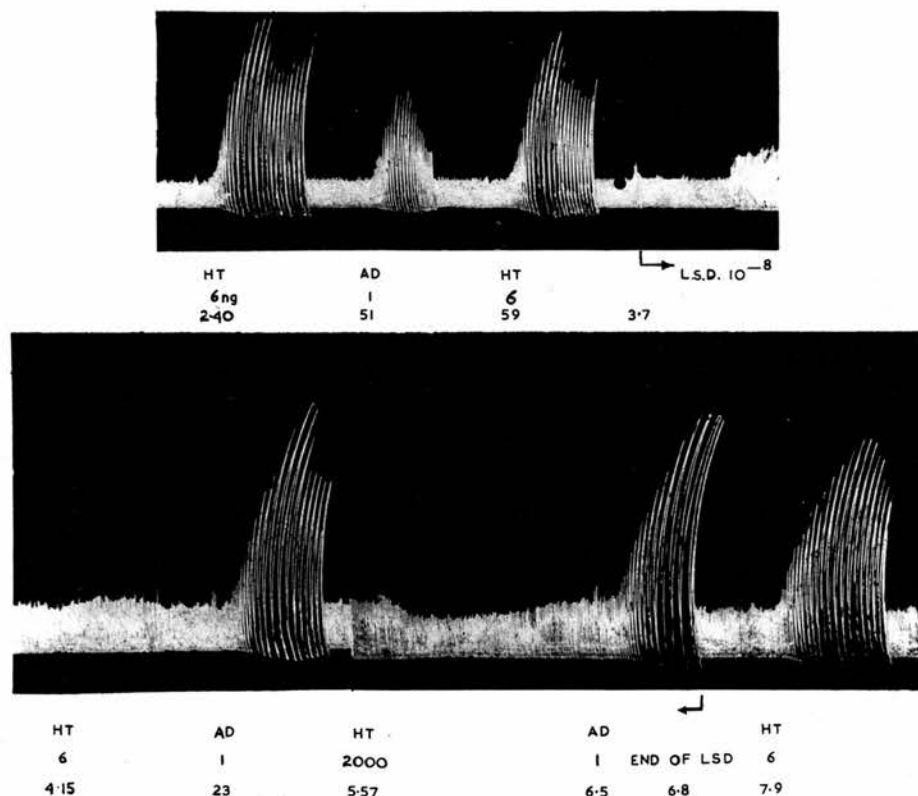
    dihydroergocornine,

    dihydroergokryptine.

Ergotamine, ergotoxine and ergometrine generally caused much spontaneous activity in the concentrations used, but in all cases there was evidence of a fall in the response to 5-hydroxytryptamine in conditions where there was no change in the response to a choline ester. However, those observations which could be made, indicated that slightly higher concentrations of ergotamine and ergotoxine as compared to dihydroergotamine, were required to inhibit the effects of 5-hydroxytryptamine while ergometrine was the least active.

Figure 7 shows that a concentration of 500 $\mu$ g/l. ergometrine produced contractions and rhythmic activity of rat's uterus. It was kept in the bath for 10 minutes and then washed out. The subsequent direct effects of 5-hydroxytryptamine and tryptamine were only partially inhibited while the effect of acetylcholine was potentiated. Higher concentrations (25 mg/l.) of ergometrine did not completely abolish the response to 5-hydroxytryptamine and tryptamine./

Figure 8



Rabbit's ear perfused as in Fig. 4. Doses in ng.

HT - 5-hydroxytryptamine

AD - adrenaline

LSD - lysergic acid diethylamide  
(10 µg/l.) specifically  
inhibited the response to HT.

tryptamine.

It was frequently observed that ergot alkaloids, especially dihydroergotamine, dihydroergokryptine, ergotamine and ergometrine in low concentrations potentiated the response of rat's uterus to acetylcholine. This result confirms that of Dale (1906).

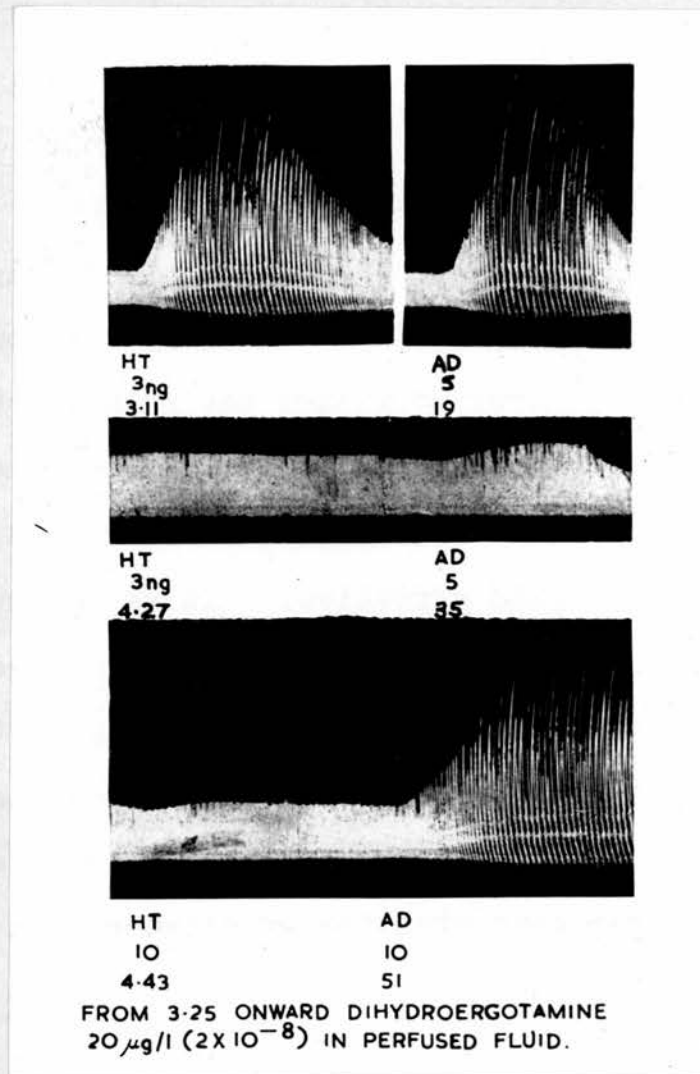
Dihydroergocristine caused marked inhibition, but this was not specific.

The results with dihydroergotamine confirm those of Erspamer (1952b; 1953a) and Fingl and Gaddum (1953).

Lysergic acid diethylamide, dihydroergotamine, ergotamine and ergometrine were also tested on the rabbit's ear. With this tissue it is possible to compare their actions against 5-hydroxytryptamine and adrenaline, both of which cause vasoconstriction. Whenever this comparison has been made, the response to 5-hydroxytryptamine has been depressed more than the response to adrenaline.

Figure 8 shows the results of an experiment where 5-hydroxytryptamine (6 ng) had more vasoconstrictor action than adrenaline (1 ng). Lysergic acid diethylamide was then perfused in a concentration of 10  $\mu$ g/l. and this abolished the constrictor effect of/

Figure 9



Rabbit's ear perfused as in Fig. 4.

Top tracing: Control doses of 5-hydroxytryptamine (3ng) and adrenaline (5ng).

Middle and bottom tracing: Dihydroergotamine antagonised both drugs but 5-hydroxytryptamine more than adrenaline.

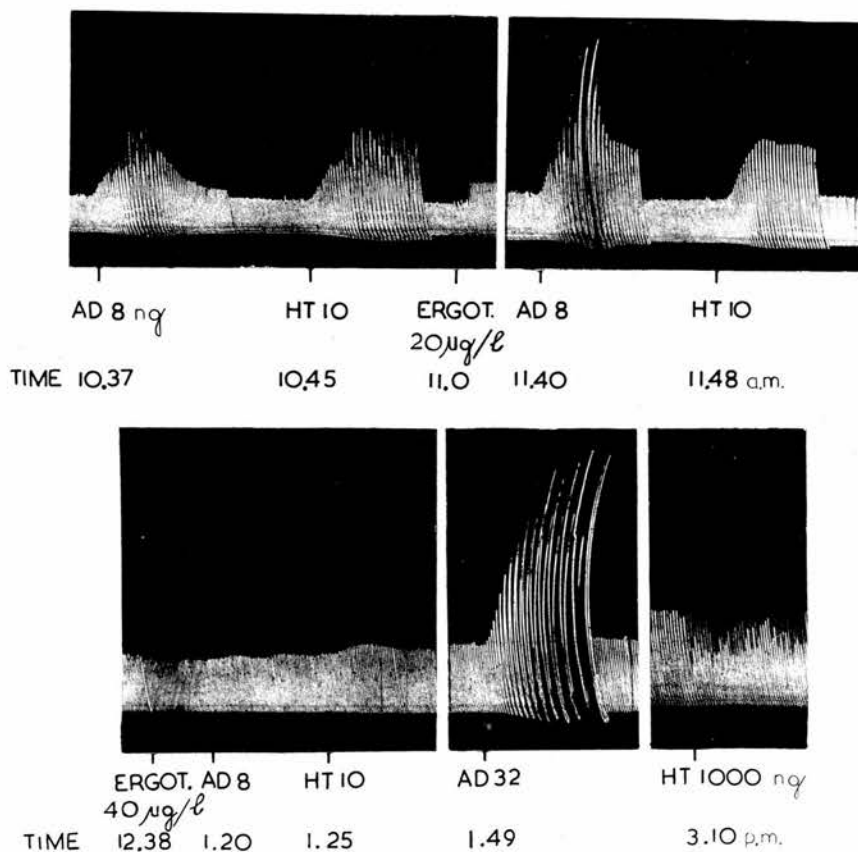


of 5-hydroxytryptamine even when the dose was increased to 2,000 ng. At the same time, the response to adrenaline showed a gradual increase. Again, when the ordinary solution was perfused for an hour, the response to 5-hydroxytryptamine returned almost to its original value.

Figure 9 shows the results of a similar experiment with dihydroergotamine. A concentration of 20  $\mu\text{g}/\text{l.}$  of this drug abolished the response to 5-hydroxytryptamine and greatly diminished that to adrenaline, but when the doses were increased, adrenaline was again effective. It was evident that dihydroergotamine was more active as an antagonist to 5-hydroxytryptamine than as an antagonist to adrenaline, but its action was not so specific as that of lysergic acid diethylamide. Again, a slightly higher concentration of dihydroergotamine was required to inhibit the effect of 5-hydroxytryptamine.

In a concentration of 20  $\mu\text{g}/\text{l.}$  ergotamine did not produce any significant inhibition of the response to 5-hydroxytryptamine. In a concentration of 40  $\mu\text{g}/\text{l.}$  the responses to both 5-hydroxytryptamine and adrenaline were abolished, but a 4-fold dose of adrenaline was effective while a 100-fold dose of/

Figure 10



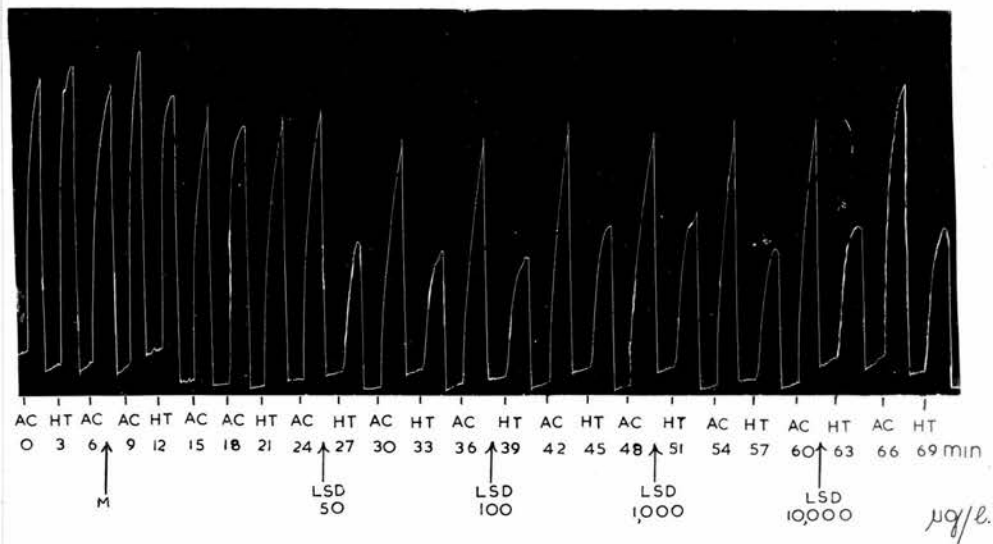
Rabbit's ear perfused as in Fig. 4.

AD - adrenaline

HT - 5-hydroxytryptamine

Ergot - Ergotamine (20 µg/l.) had no effect. In a higher concentration (40 µg/l.) ergotamine antagonised both drugs, but HT more than adrenaline.

Figure 11



Guinea-pig's ileum - 2 ml. bath

AC - acetylcholine 7 ng

HT - 5-hydroxytryptamine 130 ng

M - mepyramine (100 µg/l.) was present in the Tyrode solution from 7 min.

LSD - lysergic acid diethylamide caused partial inhibition of the response to HT.

of 5-hydroxytryptamine was not (Fig. 10).

The inhibitory activity of these ergot alkaloids on the rabbit's ear developed gradually and continued to increase with time as in the experiments with rat's uterus.

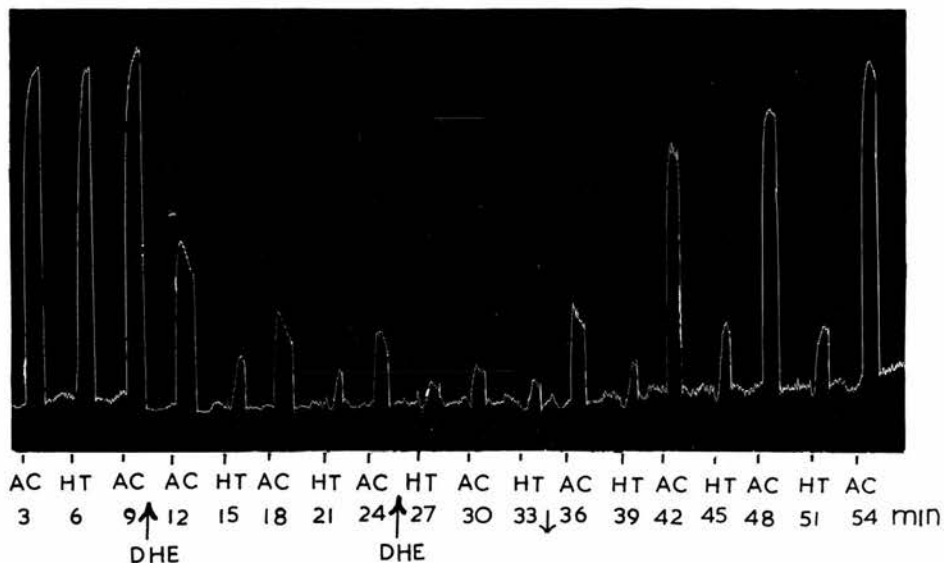
Ergometrine in a concentration of 20-100  $\mu\text{g}/\text{l}$ . had no significant effect on the response to 5-hydroxytryptamine or adrenaline.

All these ergot alkaloids produced slight vasoconstriction of the rabbit's ear in the concentration used.

The antagonism between 5-hydroxytryptamine and the ergot alkaloids on the guinea-pig's ileum is comparatively feeble and less clearly specific. In a concentration of 100  $\mu\text{g}/\text{l}$ . lysergic acid diethylamide reduced the response to 5-hydroxytryptamine by 50 per cent but higher concentrations (10,000  $\mu\text{g}/\text{l}$ .) appeared to have no more effect (Fig. 11). The response to histamine and acetylcholine were not altered in these experiments. Lysergic acid diethylamide in concentrations greater than 100  $\mu\text{g}/\text{l}$ . and occasionally even at this concentration, produced contraction of the gut and interfered with the result.

If/

Figure 12



Guinea-pig's ileum - 2 ml. bath

Mepyramine 100  $\mu$ g/l. in Tyrode solution.

AC - acetylcholine 15 ng

HT - 5-hydroxytryptamine 150 ng

DHE - dihydroergotamine (5 mg/l. 10-25 min. and 10 mg/l. 26-34 min) inhibited responses to HT and acetylcholine. After dihydroergotamine was removed from the bath the response to acetylcholine recovered gradually to its original level but there was slight recovery of the response to HT.

If mepyramine in a concentration of 100  $\mu\text{g}/\text{l}$ . was present in the bath fluid, no such stimulation of the gut was observed after higher doses of lysergic acid diethylamide. It could be argued that lysergic acid diethylamide liberates histamine in the guinea-pig's gut which was the cause of stimulation. The evidence for such an assumption is very meagre.

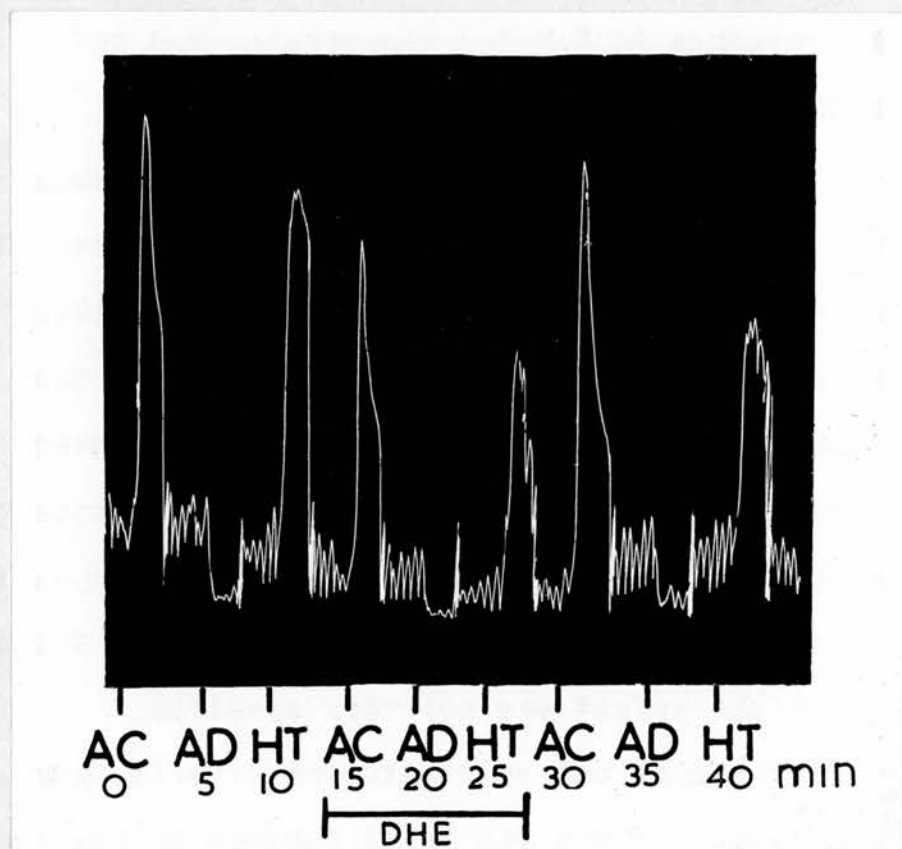
Low concentrations of dihydroergotamine had no effect but when higher concentrations (2,500-25,000  $\mu\text{g}/\text{l}$ .) were used the response to 5-hydroxytryptamine was depressed. In these conditions, the response to histamine was also depressed, sometimes even more than that to 5-hydroxytryptamine.

Figure 12 shows that dihydroergotamine (5-10mg/1.) nearly completely blocked the response to 5-hydroxytryptamine and markedly inhibited the response to acetylcholine. After the drug was washed out the response to 5-hydroxytryptamine showed only partial recovery while the response to acetylcholine recovered completely. Mepyramine (100 $\mu\text{g}/\text{l}$ .) was present in the Tyrode solution throughout the experiment.

Dihydroergotamine thus seemed to be less specific than lysergic acid diethylamide on the guinea-pig's ileum.

Dihydroergotamine/

Figure 13



Rabbit's duodenum - 13 ml. bath

AC - acetylcholine 1  $\mu$ g

AD - adrenaline 3  $\mu$ g

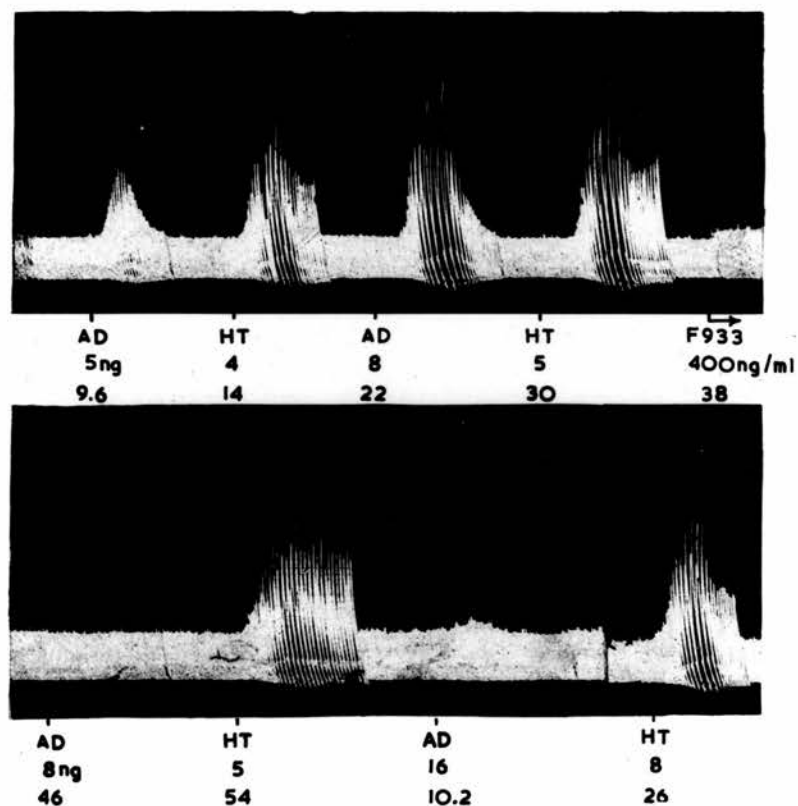
HT - 5-hydroxytryptamine 10  $\mu$ g

9  
DHE - dihydroergotamine (3.84mg/l.)  
during time signal produced more inhibition of the response to HT than of that to acetylcholine. The response to adrenaline was also inhibited.



Dihydroergotamine inhibits the response of rabbit's duodenum to 5-hydroxytryptamine. Figure 13 shows that dihydroergotamine in a concentration of 50  $\mu$ g in a 13 ml bath produced about 39% inhibition of the response to 5-hydroxytryptamine and 20% inhibition of the response to acetylcholine. Increasing the concentration of dihydroergotamine to 100  $\mu$ g in the bath almost completely suppressed the effect of 5-hydroxytryptamine, but the inhibition of the response to acetylcholine remained about the same (19%). When dihydroergotamine was removed, both responses recovered. Thus the contractions of the rabbit's duodenum induced by 5-hydroxytryptamine were blocked by dihydroergotamine (Fingl and Gaddum, 1953) but the effect was not very specific. Dihydroergotamine had no excitatory effects upon the duodenum. There was no difference in the action of dihydroergotamine in normal Locke's solution and in Locke's solution containing reduced calcium and glucose concentrations as employed for the experiments with the rat's uterus. The inhibitory response to adrenaline seemed to be also suppressed but its extent could not be accurately judged as there/

Figure 14



Rabbit's ear perfused as in Fig. 4.

AD - adrenaline

HT - 5-hydroxytryptamine

F933 - (Piperoxane) abolished  
the response to adrenaline  
without much effect on the  
response to HT.

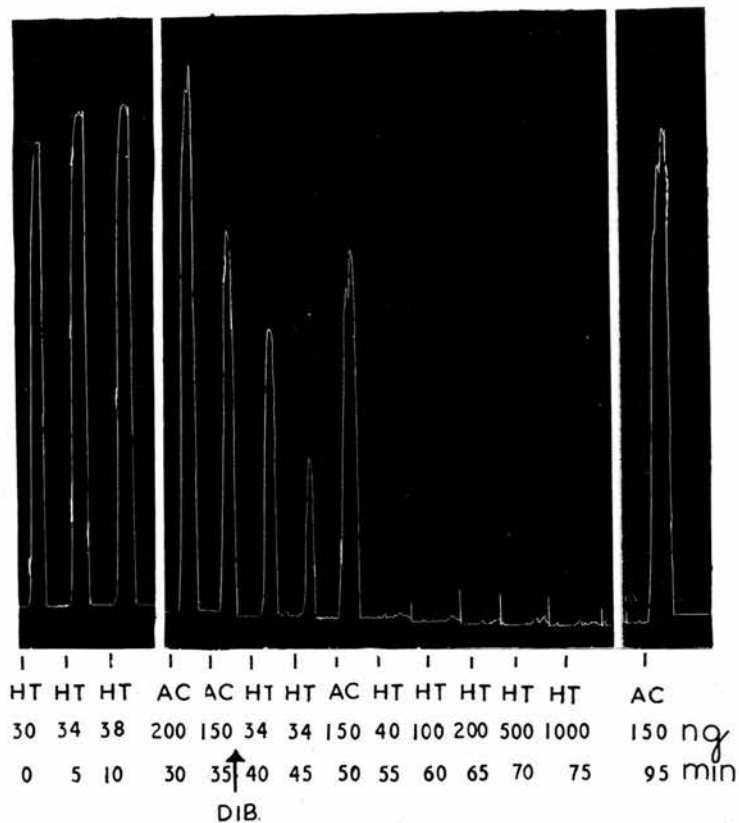
there was a fall in the tone of the muscle after the addition of dihydroergotamine in the bath.

Piperoxane (F933, piperidylmethylbenzodioxane).

Piperoxane is known as an antagonist of adrenaline, but it had no significant antagonistic action to 5-hydroxytryptamine in concentrations up to 50 mg/l. on the rat's uterus (Fingl and Gaddum, 1953; Erspamer, 1953a). High concentrations (25-50 mg/l.) of piperoxane caused the appearance of rhythmic contractions which prevented observations. However, in these experiments, no evidence of 5-hydroxytryptamine blockade was apparent when piperoxane had been removed from the bath after 10 minutes contact and rhythmic activity had ceased.

It had no anti-5-hydroxytryptamine effect on the rabbit's ear (Fingl and Gaddum, 1953). Figure 14 shows the results of an experiment in which 5-hydroxytryptamine was first compared with adrenaline and found to be 1.5 times as active. The perfusion fluid was then replaced by a similar fluid containing piperoxane (400 µg/l.). This abolished the response to adrenaline without any definite effect on the response to 5-hydroxytryptamine. It is clear that antagonism on the rabbit's ear to adrenaline and

Figure 15



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine

AC - acetylcholine

Dib.- dibenamine (13  $\mu$ g/l.)  
specifically inhibited  
the response to HT.

5-hydroxytryptamine may vary independently. Lysergic acid diethylamide is more active against 5-hydroxytryptamine and piperoxane is more active against adrenaline.

Dibenamine (N,N-dibenzyl- $\beta$ -chloroethylamine) is known as an antagonist of adrenaline with a very prolonged action. It also has some antihistamine activity (Nickerson, 1949; Fleckenstein, 1952). It antagonised the action of 5-hydroxytryptamine on all of the three tissues used in the present experiment.

Figure 15 shows the results of an experiment on the rat's uterus in which dibenamine in a concentration of 13  $\mu\text{g}/\text{l}$ . abolished the effect of 5-hydroxytryptamine, even when the dose of 5-hydroxytryptamine was increased to 1,000 ng, without affecting the response to acetylcholine. Concentrations as low as 5  $\mu\text{g}/\text{l}$ . of dibenamine greatly reduced the response of rat's uterus to 5-hydroxytryptamine. The effect continued to increase for an hour or more. A concentration of 100  $\mu\text{g}/\text{l}$ . abolished the response to 5-hydroxytryptamine and slightly reduced that to acetylcholine. The response to 5-hydroxytryptamine was immediately, completely and irreversibly, blocked. The quantitative estimation of its anti-5-hydroxytryptamine/

tryptamine effect on rat's uterus will be discussed under Section IV.

The receptors in the rabbit's ear were less sensitive to dibenamine. A concentration of 100  $\mu\text{g}/\text{l.}$  caused 75% inhibition of the response to adrenaline and 64% inhibition of the response to 5-hydroxytryptamine. As in the case of the rat's uterus, the block developed gradually for an hour or so. Even with higher concentrations of dibenamine, adrenaline effects were slightly more inhibited than 5-hydroxytryptamine effects. Both antagonisms persisted when the dibenamine was washed away.

Guinea-pig's ileum was even less sensitive than the rabbit's ear. Dibenamine (1000  $\mu\text{g}/\text{l.}$ ) reduced the responses to histamine and 5-hydroxytryptamine about equally (52%) without affecting that to acetylcholine. When the dibenamine was washed away the response to 5-hydroxytryptamine returned, but not that to histamine.

Dibenamine was thus found to be a powerful antagonist for 5-hydroxytryptamine on the rat's uterus (Erspamer, 1952b; 1953a). On the other two tissues its action against 5-hydroxytryptamine was feeble/

feebler, and less than its action against adrenaline and histamine (Fingl and Gaddum, 1953). In contrast to the results with the rat's uterus and rabbit's ear, the partial inhibition of the effect of 5-hydroxytryptamine on intestinal tissue by ergot alkaloids and dibenamine was immediate and did not increase with time.

SY-28 (N-ethyl-N-1-naphthylmethyl-2-bromoethylamine). This is known as an antagonist of adrenaline and histamine (Graham and Lewis, 1953).

A concentration of 10  $\mu\text{g}/\text{l.}$  of SY-28 potentiated the response to 5-hydroxytryptamine on the rat's uterus and a concentration of 50-100  $\mu\text{g}/\text{l.}$  inhibited the effect of 5-hydroxytryptamine markedly. The response to acetylcholine was also reduced, but less than the 5-hydroxytryptamine effect. As with dibenamine, the block developed gradually for an hour or so. It was less active and less specific than dibenamine for its anti-5-hydroxytryptamine action on the rat's uterus.

SY-28 (50  $\mu\text{g}/\text{l.}$ ) suppressed the vasoconstrictor effects of 5-hydroxytryptamine and adrenaline on the rabbit's ear to the same marked extent. The effect continued to increase indefinitely and was irreversible.

The/



The effect of SY-28 on guinea-pig's ileum was non-specific. A concentration of 50  $\mu\text{g/l}$ . produced 20% inhibition of the response to 5-hydroxytryptamine and 85% inhibition of the response to histamine. It was thus more active against histamine than against 5-hydroxytryptamine.

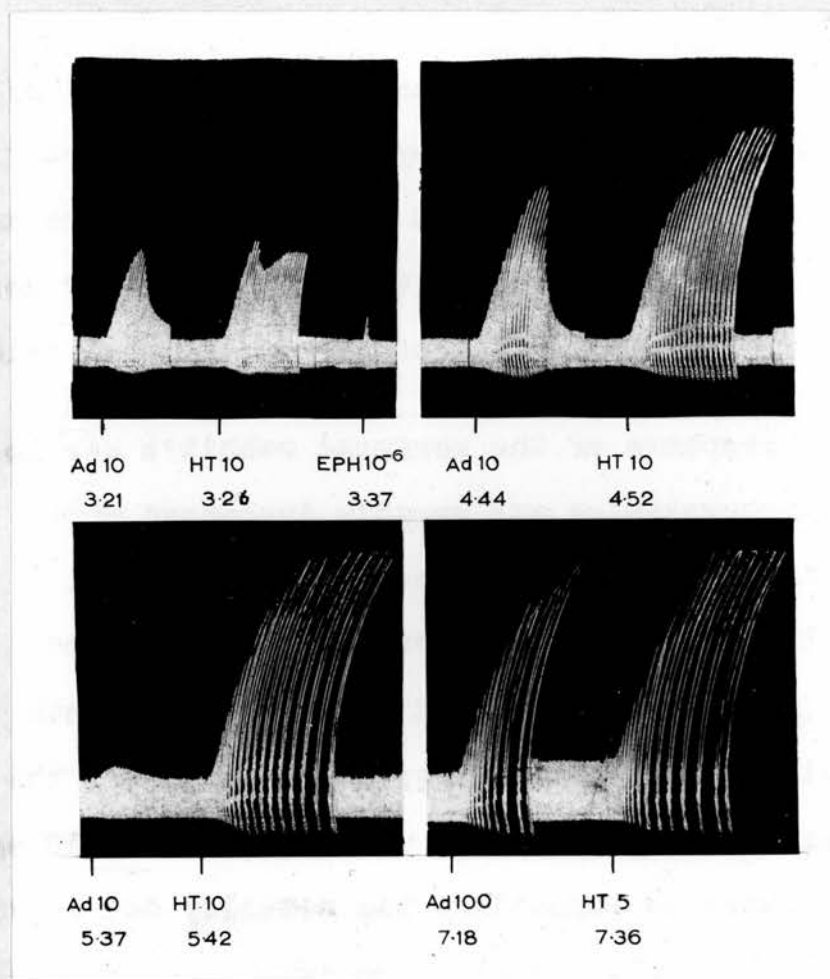
When the drug was removed from the bath the responses to 5-hydroxytryptamine and histamine showed partial recovery.

The antagonism of SY-28 and 5-hydroxytryptamine on the three tissues was clearly unspecific.

Freyburger et al. (1952) produced a partial decrease of the 5-hydroxytryptamine induced hypertension in the dog with SY-28, while McCawley et al. (1952) demonstrated that the sinus tachycardia evoked in anaesthetised dogs by 5-hydroxytryptamine could not be prevented with SY-28.

Inhibitors of amine oxidase. In low concentrations ephedrine potentiates some of the actions of adrenaline and in higher concentrations it antagonises them (Gaddum and Kwiatkowski, 1938). The former action has been attributed to the inhibition of amine oxidase, which ephedrine is known to cause and since 5-hydroxytryptamine is destroyed by amine oxidase/

Figure 16



Rabbit's ear perfused as in Fig. 4. Doses in ng

AD - adrenaline

HT - 5-hydroxytryptamine

EPH - ephedrine (1 mg/l.; 3.37-5.7) increased the responses to adrenaline and HT. In a higher concentration (10 mg/l.; 5.7-7.50) ephedrine diminished the response to adrenaline, but increased the response to HT still further.

oxidase (Blaschko, 1952; Freyburger et al. 1952), it was thought possible that some of its effects might be increased by ephedrine and allied drugs. Experiments with ephedrine and amphetamine on the rat's uterus and the guinea-pig's ileum did not show any significant action of this kind. A range of doses from 1  $\mu\text{g}/\text{l.}$  to 500  $\mu\text{g}/\text{l.}$  of both drugs were tried.

The response of the perfused rabbit's ear to 5-hydroxytryptamine was greatly increased when ephedrine was present in the perfusion fluid. Ephedrine in a concentration of 1  $\text{mg}/\text{l.}$  in the perfusion fluid potentiated the effects of both adrenaline and 5-hydroxytryptamine, but when the concentration of ephedrine was increased to 10  $\text{mg}/\text{l.}$  the response to adrenaline was actually depressed while the response to 5-hydroxytryptamine was markedly increased (Fig. 16). The potentiation of the effect of 5-hydroxytryptamine is presumably due to inhibition of the amine oxidase. The inhibition of the response to adrenaline by higher concentrations of ephedrine has been attributed to blockade/

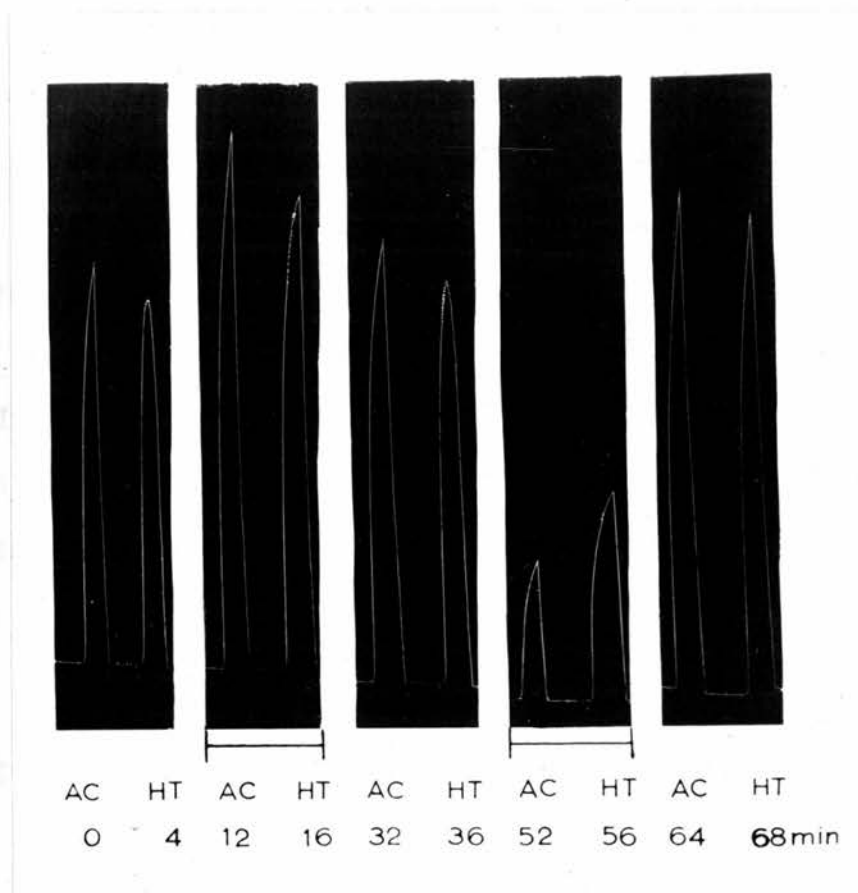
blockade of the adrenaline receptors (Gaddum and Kwiatkowski, 1938). The tryptamine receptors do not appear to be blocked by the concentrations of ephedrine used in these experiments.

The theory that this potentiation of the response to 5-hydroxytryptamine is due to inhibition of amine oxidase was confirmed by the results of one experiment with choline-p-tolyl ether (Hey, 1952; Brown and Hey, 1952). This drug, which is known to inhibit amine oxidase markedly, was added to the fluid perfused in a rabbit's ear in a concentration of 1 mg/l. This increased the effects of both adrenaline and 5-hydroxytryptamine, a higher concentration (10 mg/l.) decreased both effects.

Choline-p-tolyl ether (1  $\mu$ g-500  $\mu$ g/l.) like ephedrine and amphetamine, did not show any significant effect of this nature on the rat's uterus and the guinea pig's ileum.

Cocaine./

Figure 17



Rat's uterus - 2 ml. bath

AC - acetylcholine 500 ng

HT - 5-hydroxytryptamine 10 ng

Cocaine (10 mg/l.; 5-17 min) increased the responses to acetylcholine and HT. A higher concentration of cocaine (50 mg/l.; 37-57 min) decreased both these effects.

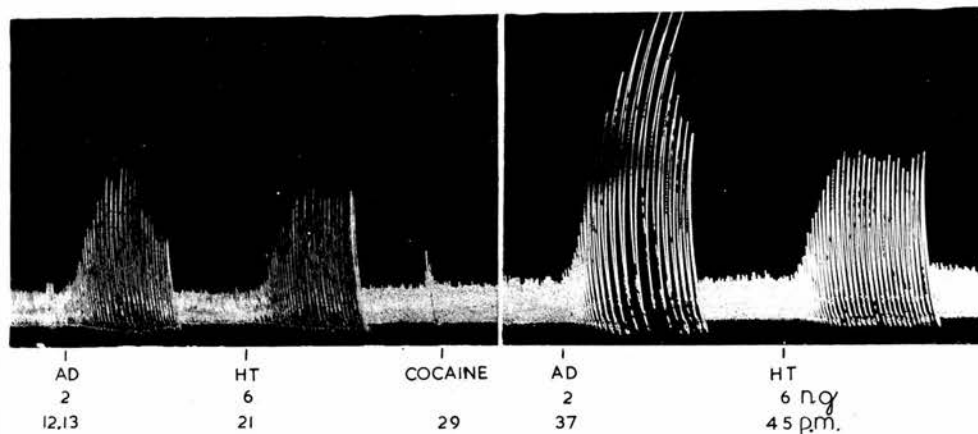
Cocaine.

Cocaine is not only a local anaesthetic but also an inhibitor of amine oxidase (Philpot, 1940). Reid and Rand (1952) found that it increased the effect of 5-hydroxytryptamine on a strip of sheep's carotid artery, but reported no experiments to test whether this was a specific effect or an unspecific increase in the contractile power of the muscle. Cocaine, however, did not show any effects which could be attributed, with confidence, to the inhibition of amine oxidase.

In a concentration of 5-10 mg/l. it increased the effects of both 5-hydroxytryptamine and acetylcholine on the rat's uterus. When it was washed away, the effects gradually returned to their original level. Lower concentrations had no significant action. A higher concentration, (50 mg/l.) temporarily inhibited the effects of both 5-hydroxytryptamine and acetylcholine. When the cocaine was washed away the effects were increased above their normal level (Fig. 17).

The action of 5-hydroxytryptamine on the rabbit's/

Figure 18



Rabbit's ear perfused as in Fig. 4

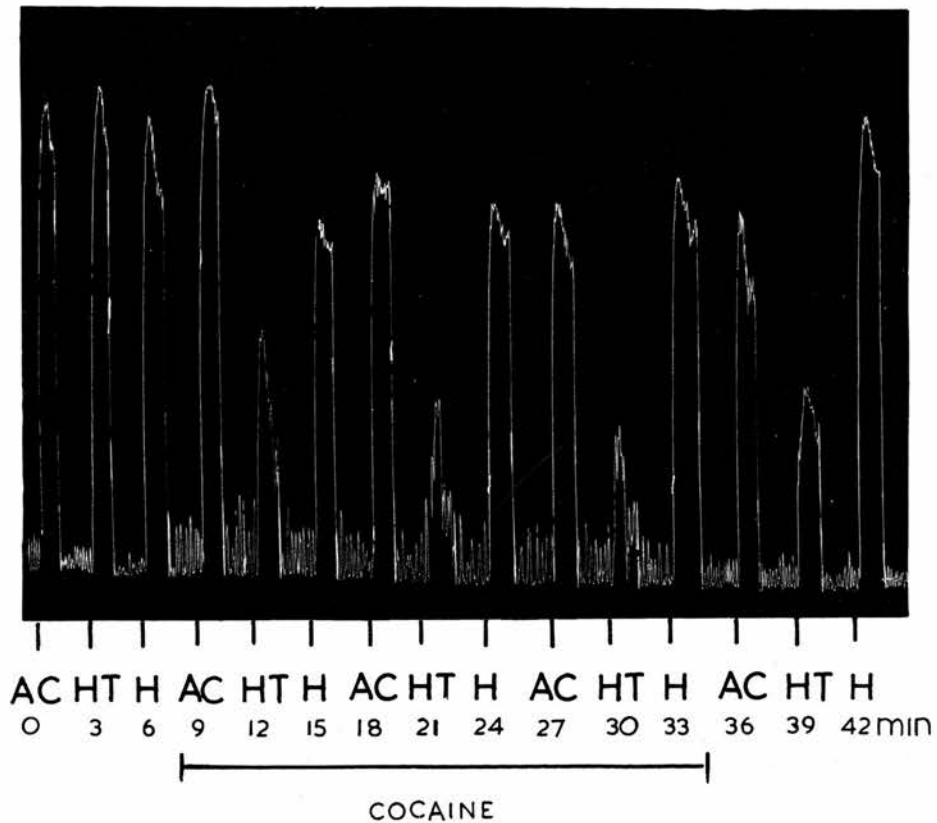
AD - adrenaline

HT - 5-hydroxytryptamine

Cocaine (10 mg/l.) increased the effect of adrenaline without significant effect on the response to HT.



Figure 19



Guinea-pig's ileum - 2 ml. bath

AC - acetylcholine 4 ng

HT - 5-hydroxytryptamine 140 ng

H - histamine 7 ng

Cocaine (10 mg/1.) inhibited markedly the response to HT without much effect on the responses to acetylcholine and histamine.

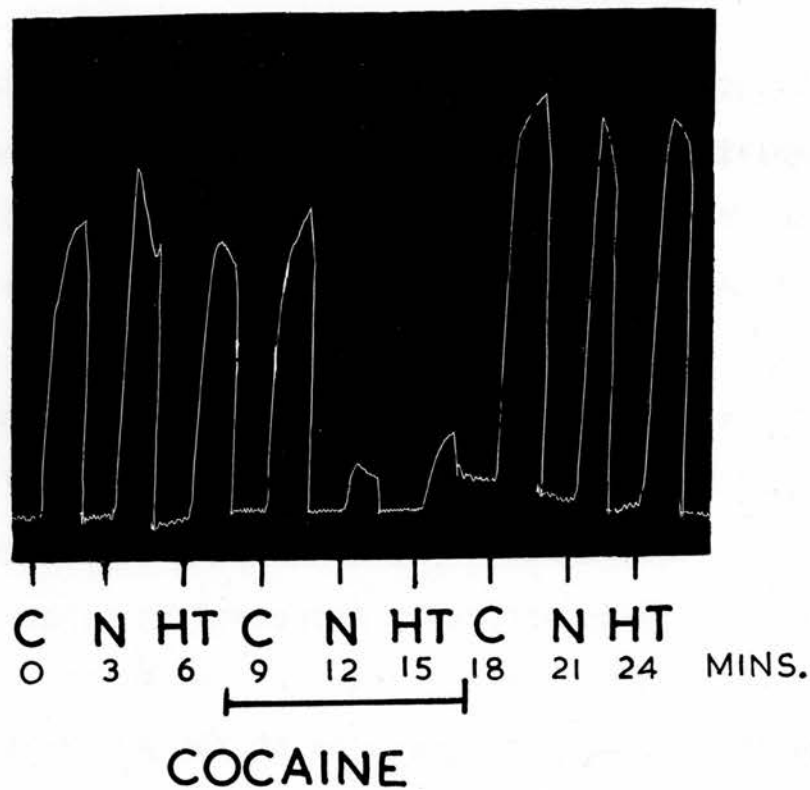
rabbit's ear was not significantly changed by cocaine (10 mg/l.) although the action of adrenaline was much increased in the same experiment (Fig. 18). The small increase in 5-hydroxytryptamine response seen in the figure is due to the increase in height of the base line caused by the slight vasoconstrictor effect of cocaine.

It has been suggested that the effect of cocaine on the response to adrenaline is due to inhibition of amine oxidase (Philpot, 1940; Blashko, 1952). If this is so, it is perhaps surprising that the cocaine did not increase the effect of 5-hydroxytryptamine in this experiment. It might be suggested that the effect of 5-hydroxytryptamine on the rabbit's ear does not depend on amine oxidase, but if so the potentiating action of ephedrine cannot be explained in the way that has been suggested above.

Cocaine (10 mg/l.) inhibited the response of the guinea-pig's ileum to 5-hydroxytryptamine reversibly without much effect on the response to histamine and acetylcholine (Fig. 19).

In another experiment (Fig. 20) the responses to 5-hydroxytryptamine and nicotine were about equally inhibited.

Figure 20



Guinea-pig's ileum - 2 ml. bath

C - carbachol 40 ng

N - nicotine 5000 ng

HT - 5-hydroxytryptamine 1000 ng

Cocaine (5 mg/l.) inhibited the responses to nicotine and 5-hydroxytryptamine without affecting the response to carbachol.

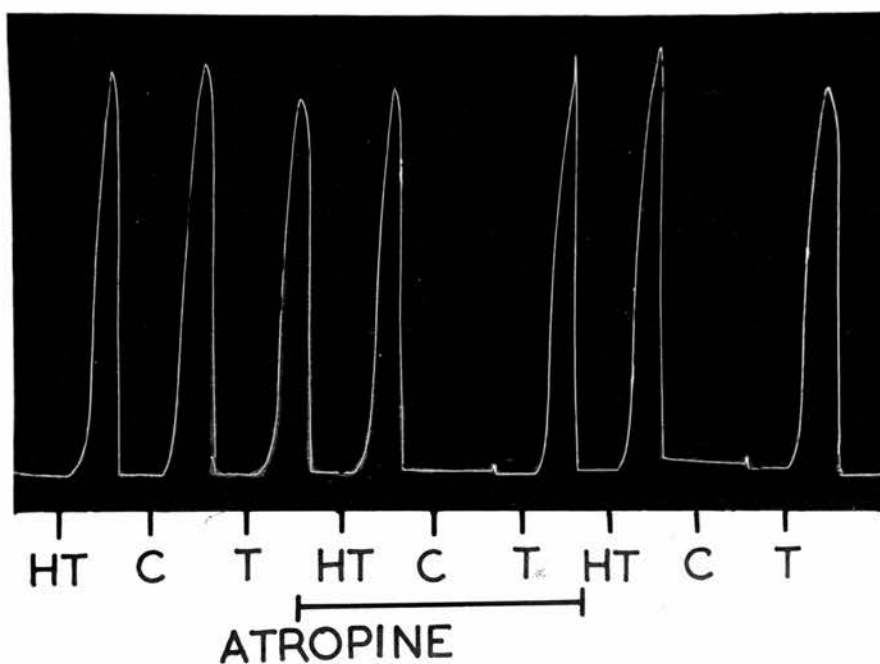
inhibited by cocaine (5 mg/l.). Both responses recovered immediately when the drug was removed from the bath.

This inhibition has been observed by others (Sinha and West, 1953; Rocha e Silva et al. 1953) and explained on the theory that 5-hydroxytryptamine acts on the nervous tissue in the intestine and that cocaine inhibits its action by paralysing these nerves.

The actions of cocaine on these three tissues were thus different. The response of the rat's uterus was unspecifically increased by low concentrations and unspecifically decreased by higher concentrations. The response of the rabbit's ear was unaffected and the response of the guinea-pig's ileum was inhibited, like the response to nicotine before the responses to histamine or choline esters. The generalisation of Sinha and West (1953) that local anaesthetics antagonise the actions of 5-hydroxytryptamine does not give all the facts.

Atropine. In concentrations of 5-100  $\mu$ g/l. atropine completely abolished the effect of choline esters on the rat's uterus, while leaving the effect of/

Figure 21



Rat's uterus - 2 ml. bath - interval 3 min.

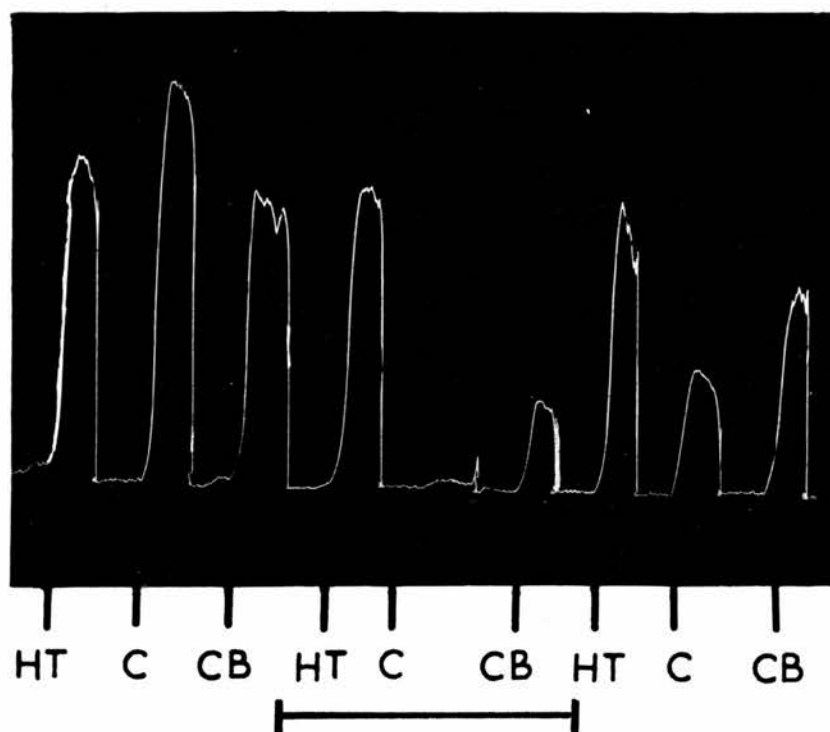
HT - 5-hydroxytryptamine 20 ng

C - carbachol 100 ng

T - tryptamine 4000 ng

Atropine (5  $\mu$ g/l. during time signal) abolished the response to carbachol but had no effect on the response to HT or tryptamine.

Figure 22



Guinea-pig's ileum - 2 ml. bath - interval 3 min.

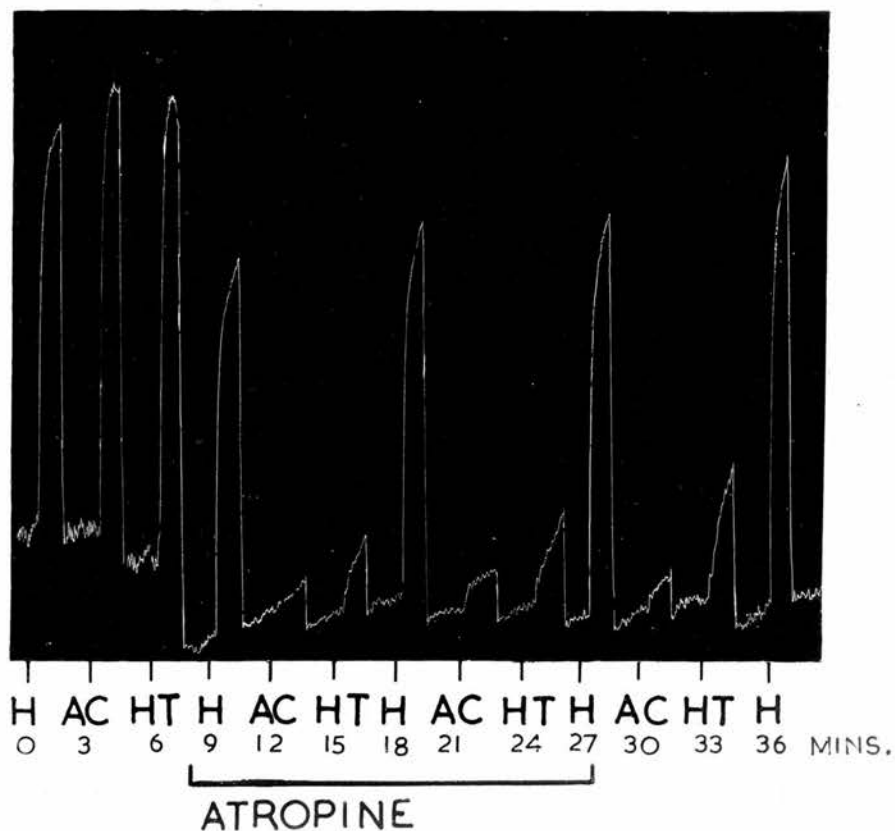
HT - 5-hydroxytryptamine 100 ng

C - carbachol 10 ng

CB - cinobufotenin 1200 ng  
(N-trimethyl-5-hydroxytryptamine)

Atropine (10 ng during time signal)  
suppressed the response to carbachol but  
not that to HT. With cinobufotenin the  
effect was intermediate.

Figure 23



Guinea-pig's ileum - 2 ml. bath

H - histamine 7 ng

AC - acetylcholine 7 ng

HT - 5-hydroxytryptamine 130 ng

Atropine (100  $\mu$ g/l.) nearly abolished the effect of acetylcholine and markedly diminished the effect of HT but did not alter the effect of histamine.

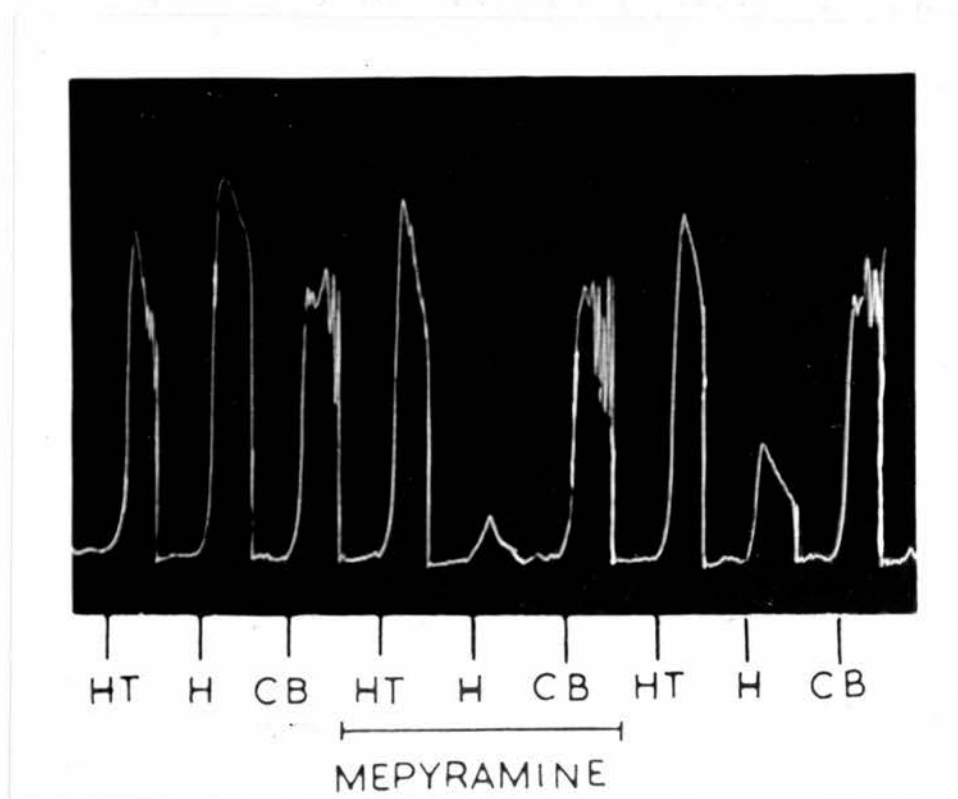


of 5-hydroxytryptamine unchanged (Fig. 21). A higher concentration (1000  $\mu\text{g/l.}$ ) diminished the effect of 5-hydroxytryptamine by 50%.

Atropine (5  $\mu\text{g/l.}$ ) abolished the response of the guinea-pig's ileum to carbachol (5  $\mu\text{g/l.}$ ) but not that to 5-hydroxytryptamine (50  $\mu\text{g/l.}$ ) (Fig. 22). The effect of cinobufotenin, (CB) was partially inhibited. Higher concentrations of atropine (100  $\mu\text{g/l.}$ ) diminished the effect of 5-hydroxytryptamine and abolished the effect of acetylcholine (3-5  $\mu\text{g/l.}$ ) while leaving that of histamine (3-5  $\mu\text{g/l.}$ ) unchanged (Fig. 23). These results confirm those of various workers (Gaddum, 1953a; Rapport and Koelle, 1953; Robertson, 1953; Rocha e Silva et al. 1953). Atropine inhibits the effect of 5-hydroxytryptamine on this tissue more than that of histamine, but less than those of choline esters. The effects of 5-hydroxytryptamine and nicotine were inhibited to about the same extent by atropine in this tissue. The actions of atropine on the guinea-pig's ileum are thus similar to those on the rat's uterus, but there was less difference between the doses effective against 5-hydroxytryptamine and acetylcholine.

Atropine/

Figure 24



Guinea-pig's ileum - 2 ml. bath - interval 3 min.

HT - 5-hydroxytryptamine	200 ng
H - histamine	10 ng
CB - cinobufotenin	1000 ng

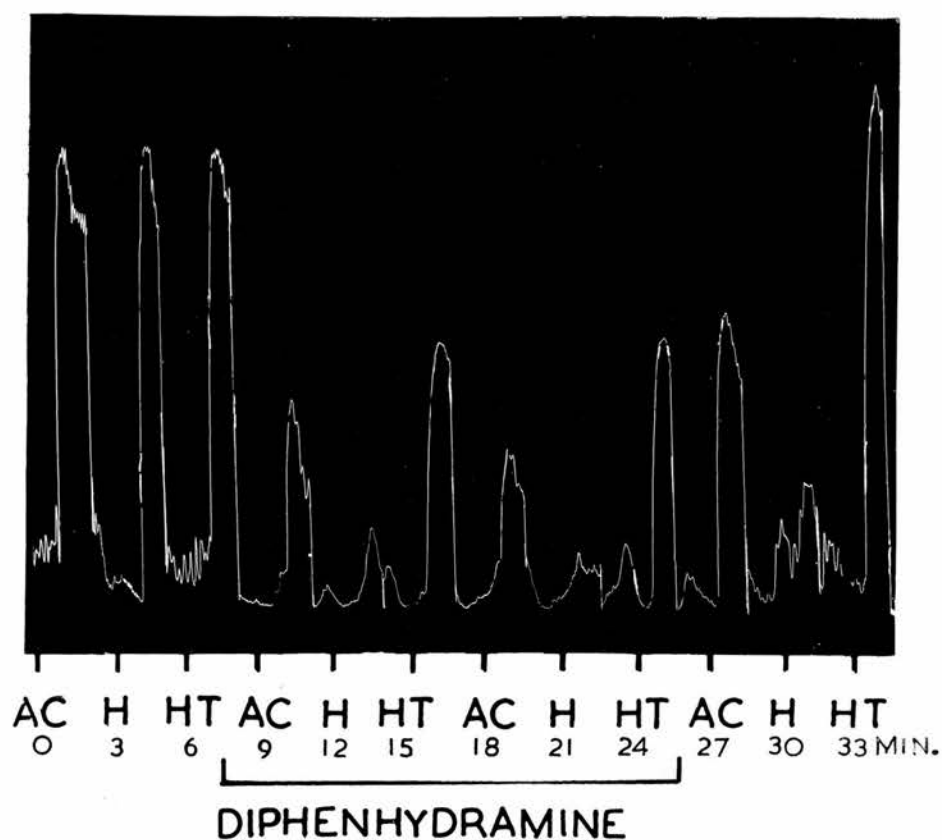
Mepyramine (5 $\mu$ g/l.) inhibited the response to histamine but not that to HT or cinobufotenin.

Atropine (100-1000  $\mu\text{g}/\text{l.}$ ) had no action on the effect of 5-hydroxytryptamine on the rabbit's ear.

Antihistamines. Experiments were done with mepyramine and diphenhydramine. The first is one of the most specific antihistamines, and the second is less specific since it has some action against acetylcholine (Schild, 1947). The effect of 5-hydroxytryptamine on the rat's uterus was partially blocked by either drug in a concentration of 100 $\mu\text{g}/\text{l.}$  and completely blocked by 1000  $\mu\text{g}/\text{l.}$  In both cases, the response to acetylcholine was inhibited. With diphenhydramine the acetylcholine response was inhibited to the same extent as the response to 5-hydroxytryptamine, while mepyramine (1000  $\mu\text{g}/\text{l.}$ ) caused only 54% inhibition of the response to acetylcholine.

Mepyramine (1000  $\mu\text{g}/\text{l.}$ ) had little or no effect on the response of the guinea-pig's ileum to 5-hydroxytryptamine or acetylcholine although a concentration of 5  $\mu\text{g}/\text{l.}$  was sufficient to abolish the response to histamine (Fig. 24). Diphenhydramine (1000  $\mu\text{g}/\text{l.}$ ) produced partial inhibition of the responses to both 5-hydroxytryptamine and acetylcholine, and abolished the response to histamine almost/

Figure 25

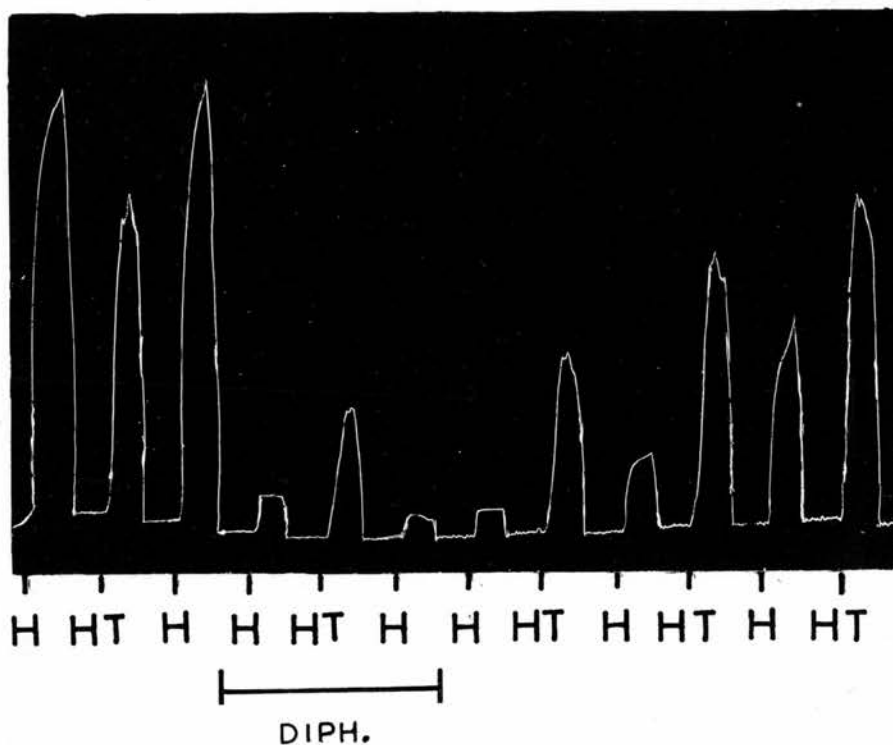


Guinea-pig's ileum - 2 ml. bath

AC - acetylcholine 10 ng  
H - histamine 13 ng  
HT - 5-hydroxytryptamine 100 ng

Diphenhydramine (1 mg/1.)  
caused partial inhibition  
of the responses to HT and  
acetylcholine but abolished  
the response to histamine  
almost completely.

Figure 26



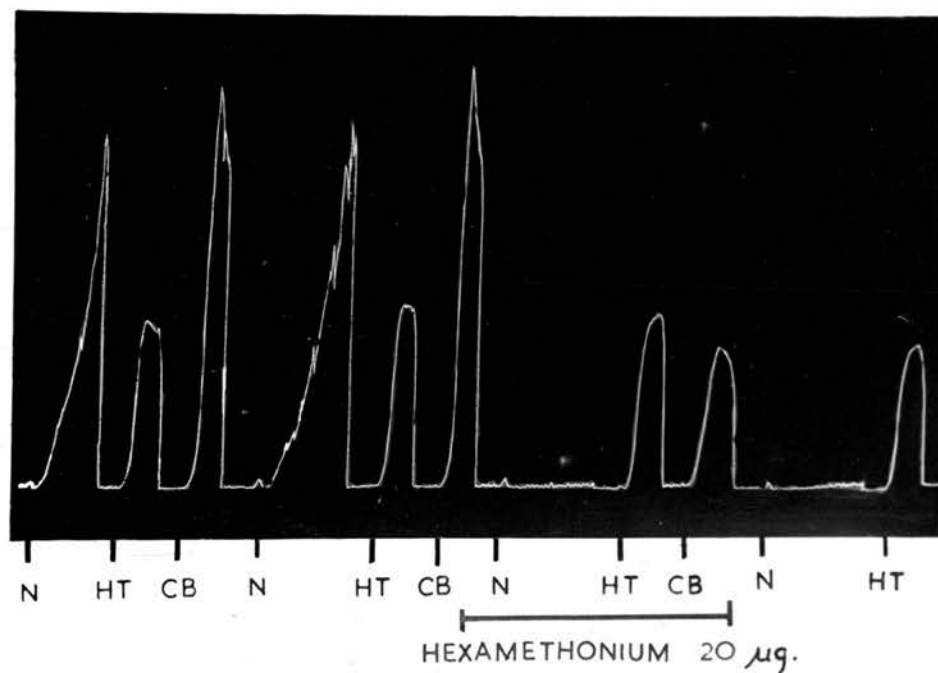
Guinea-pig's ileum - 2 ml. bath - interval 3 min.

H - histamine 10 ng

HT - 5-hydroxytryptamine 500 ng

Diph - diphenhydramine (25  $\mu$ g/l.) caused marked inhibition of the response to HT and almost completely abolished the response to histamine. After diphenhydramine was removed from the bath, the response to HT recovered earlier than that to histamine.

Figure 27



Guinea-pig's ileum - 2 ml. bath - interval 3 min.

N - nicotine	3000 ng
HT - 5-hydroxytryptamine	150 ng
CB - cinobufotenin	1500 ng

Hexamethonium abolished the response to nicotine and decreased the response to CB but had no effect on the response to HT

almost completely (Fig. 25). In another experiment (Fig. 26) diphenhydramine in a low concentration (25  $\mu$ g/l.) diminished the response to 5-hydroxytryptamine to a marked extent. After the drug was washed out, the response to 5-hydroxytryptamine recovered earlier than that due to histamine.

These two antihistamines thus produced about the same effect on the responses of both tissues to 5-hydroxytryptamine as on their responses to acetylcholine. The experiment with the guinea-pig's ileum provides another example confirming the generalisation that mepyramine is a more active and more specific antihistamine than diphenhydramine.

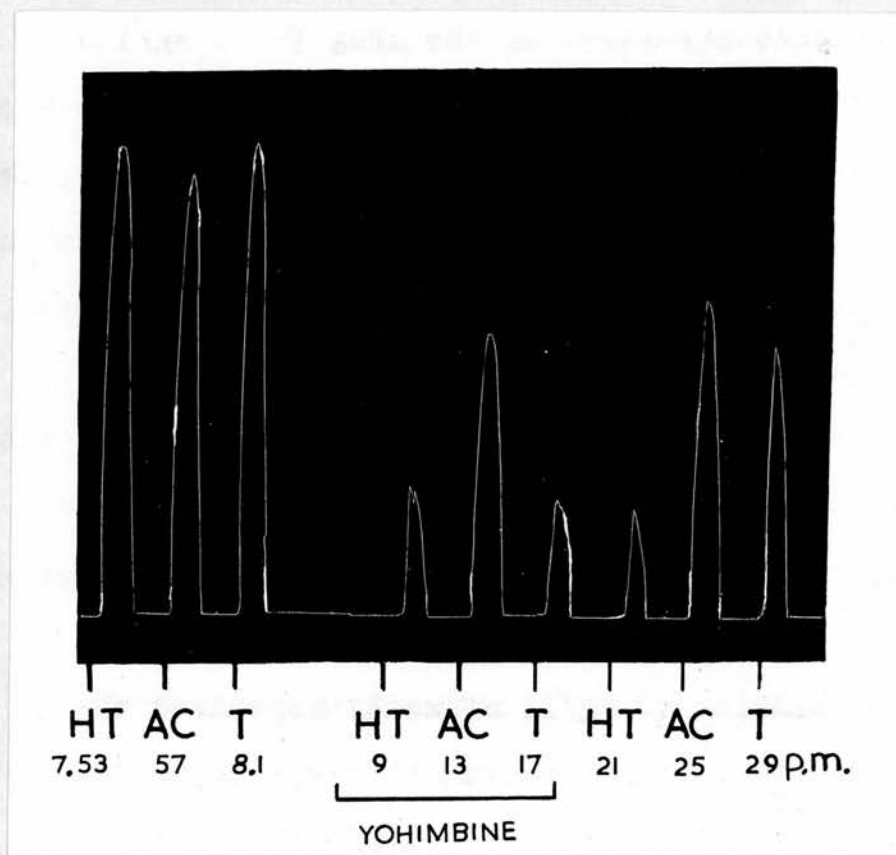
These results are in general agreement with those of Reid and Rand (1951 and 1952), Erspamer (1952b) and Rapport and Koelle (1953).

Inactive or unspecific antagonists. The following drugs showed no specific antagonism to 5-hydroxytryptamine.

Hexamethonium (10 mg/l.) inhibited the effect of nicotine on the guinea-pig's ileum without altering the effect of 5-hydroxytryptamine (Fig. 27) The response to cinobufotenin (CB) was also partially suppressed. The/



Figure 28



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine      5 ng  
AC - acetylcholine              300 ng  
T - tryptamine                  2000 ng

Yohimbine (5 mg/l.) inhibited the responses to HT and tryptamine more than that to acetylcholine.

The actions of this compound are described on P.84 This confirms the results of Robertson (1953) and Rocha e Silva et al. (1953).

Mescaline (50  $\mu$ g-50 mg/l.) did not affect the response of the rat's uterus to 5-hydroxytryptamine. Higher concentrations of the drug ( $>1$  mg/l.) caused contraction of the uterus. These were kept in the bath for 10 minutes and then washed out, when the subsequent direct effect of 5-hydroxytryptamine was not affected. Mescaline is thought, by some, to resemble lysergic acid diethylamide in its effects on the central nervous system. These two drugs might therefore have been expected to have similar effects as antagonists of 5-hydroxytryptamine but this was not the case.

Yohimbine (5 mg/l) markedly depressed the response of the rat's uterus to 5-hydroxytryptamine and tryptamine (Fig.28). The response to acetylcholine was also depressed though less, and the antagonism was thus not very specific. Low concentrations (500  $\mu$ g/l.) had no effect on the responses to 5-hydroxytryptamine and tryptamine. The antagonism between yohimbine and tryptamine on the blood pressure of dog was described by Raymond-Hamet (1941).

(1941). The antagonism of yohimbine and 5-hydroxytryptamine on isolated strips of carotid artery has been described by Reid and Rand (1952) and Shaw and Woolley (1953). These authors obtained effects with concentrations as low as 0.1 mg/l. but give no evidence that the inhibition was specific. Erspamer (1953a) however, reported that 1 mg/l. of yohimbine did not affect the response to 5-hydroxytryptamine on the rat's uterus, but 10 mg/l. showed a consistent depressive effect on the response to 5-hydroxytryptamine without any effect on the response to acetylcholine.

Physostigmine in low concentrations (100-1000  $\mu$ g/l.) caused a small increase in the response of the rat's uterus to 5-hydroxytryptamine and higher concentrations (10-50 mg/l.) sometimes caused a decrease, but the effects were feeble.

Although both yohimbine and eserine contain an indole ring there is, as yet, no evidence that either of them is a specific antagonist of 5-hydroxytryptamine.

Adrenaline. In concentrations of 1-100  $\mu$ g/l. adrenaline/

adrenaline caused marked inhibition of the effect of tryptamine on rat's uterus. The effect was un-specific, as carbachol was likewise inhibited. The effect of adrenaline on the guinea-pig's ileum was not tested. Rocha e Silva et al. (1953) reported that adrenaline (333-334  $\mu\text{g}/\text{l.}$ ) caused marked inhibition of the response of the guinea-pig's ileum to 5-hydroxytryptamine. The responses to histamine and acetylcholine were also depressed by adrenaline.

Tyramine. In concentrations of 500-1000  $\mu\text{g}/\text{l.}$  tyramine did not inhibit the response of the rat's uterus to tryptamine, but the effect of carbachol was strongly suppressed. Tyramine did not cause stimulation of the uterus in the doses used. Its effect on the guinea-pig's ileum was not tested.

Erspamer (1952b; 1953a) reported that adrenaline strongly inhibited the uterus stimulating action of enteramine, but the other sympathomimetic drugs (tyramine, norsynephrine, noradrenaline) counter-acted enteramine to a much less degree or were practically ineffective.

#### Discussion/

Discussion.

Experiments with specific antagonists confirm the conclusion that 5-hydroxytryptamine produces some of its effects by acting on specific receptors. With mepyramine, piperoxane and atropine it is possible to abolish the effects of histamine, adrenaline and acetylcholine respectively, without altering the effects of 5-hydroxytryptamine. Higher concentrations of these drugs may antagonise 5-hydroxytryptamine, but this does not invalidate the evidence given by the experiments with low concentrations which show that some at least of the effects of 5-hydroxytryptamine are due to combination with receptors which are not identical with the receptors for histamine, adrenaline or acetylcholine.

The drugs which do antagonise 5-hydroxytryptamine have given different results when tested on different tissues. Lysergic acid diethylamide is a very active and specific antagonist for 5-hydroxytryptamine in experiments on the rat's uterus or the rabbit's ear, but had little effect in experiments/

experiments on the guinea-pig's ileum even when high concentrations were used. Other ergot alkaloids and gramine gave similar though less striking results. These drugs were more effective on the rat's uterus and the rabbit's ear than on the guinea-pig's ileum, but the reverse is true of atropine, cocaine, or excess of 5-hydroxytryptamine itself.

Other workers (Robertson, 1953; Rocha e Silva et al. 1953) have obtained similar results with the last three drugs on guinea-pig's ileum, and have suggested a theory to account for them. The effect of 5-hydroxytryptamine on this tissue resembles that of nicotine in that both effects are blocked by similar concentrations of atropine and cocaine. It is therefore suggested that 5-hydroxytryptamine acts, like nicotine, at some point in the nervous tissue in the intestine. Hexamethonium, or sufficient excess of nicotine, blocks the response to small doses of nicotine without blocking the response to 5-hydroxytryptamine. These facts are explained on the theory that 5-hydroxytryptamine acts on the post-ganglionic nerve fibres while nicotine acts on the nerve cells.

This/



This theory does not provide a satisfactory explanation of the observation of Rocha e Silva et al. (1953), that when the guinea-pig's ileum is desensitized to 5-hydroxytryptamine by excess of 5-hydroxytryptamine itself, it still gives a normal response to nicotine. If the site of action of 5-hydroxytryptamine is peripheral to that of nicotine and is paralysed, it is surprising that the response to nicotine is normal. Nicotine and 5-hydroxytryptamine appear to behave similarly to one another but independently; if the bath contains excess of either of these drugs the muscle gives no response to that drug, but a normal response to the other drug. There is nothing to suggest an anatomical difference between the sites of action of the two drugs. It is possible that the ganglion cells in the intestine contain two types of receptor, one of which is stimulated by acetylcholine or nicotine and inhibited by excess of nicotine or hexamethonium, while the other type is stimulated by 5-hydroxytryptamine and inhibited by excess of 5-hydroxytryptamine. These cells would then be comparable with various plain muscle cells which are believed to have specific receptors for/



for both histamine and acetylcholine. It is also possible that there are two types of cell, one of which is stimulated by nicotine and the other by 5-hydroxytryptamine.

Whatever may be the explanation of the behaviour of the guinea-pig's ileum, it is clear that the other two tissues behave differently. The results can be explained on the theory that there are two types of tryptamine receptors.

- A. Receptors in the plain muscle of the rat's uterus and rabbit's ear which are easily paralysed by lysergic acid diethylamide but not so easily paralysed by excess of 5-hydroxytryptamine.
- B. Receptors in the ganglia of the intestine which are easily paralysed by excess of 5-hydroxytryptamine but not easily paralysed by lysergic acid diethylamide.

If this theory is correct, the relation between lysergic acid diethylamide and 5-hydroxytryptamine is similar to the relation between acetylcholine and atropine. In both cases, the receptors in smooth muscle are more easily inhibited by the antagonist, and the receptors in the nervous tissue are more easily desensitized by excess of the active drug.

SECTION IV.

ACTION OF RELATED INDOLE COMPOUNDS.

Preliminary study of indole compounds.

In the preliminary study, the following indole compounds were tested on the rat's uterus and the guinea-pig's ileum especially for their antagonism to the effects of 5-hydroxytryptamine and tryptamine; gramine, cinobufotenin, viridobufotenin, marinobufotenin, indole, 2-methyl indole, 3-methyl indole (skatole), 7-methyl indole, 3-indolyl-acetic acid, methyl-3-indolyl acetate, indole propionic acid, indole butyric acid, isatin, isatin- $\beta$ -oxime, tryptophan, hypaphorine, adrenochrome and adrenolutine.

The actions of tryptamine bear a close resemblance to those of 5-hydroxytryptamine (Reid, 1951; Gaddum, 1953a). It is however, very much less active than 5-hydroxytryptamine, 200-500 times less active on the rat's uterus and 300 times or more, less active on the guinea-pig's ileum. The drugs which antagonised 5-hydroxytryptamine, also antagonised tryptamine.

Guinea-pig's ileum can be specifically desensitized to tryptamine by exposure to high concentrations of/

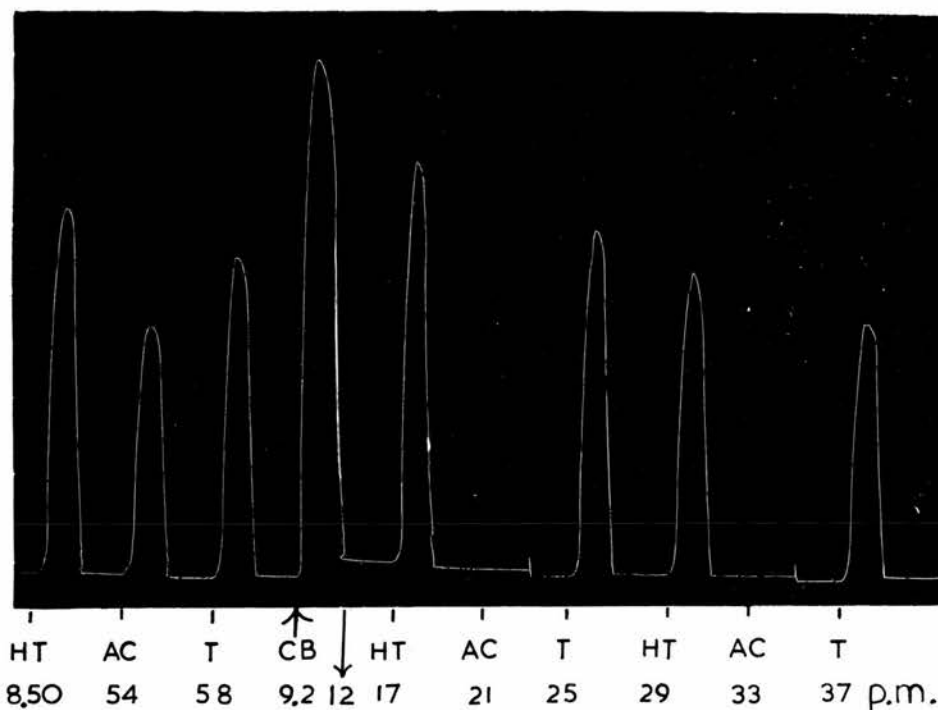
of this drug or 5-hydroxytryptamine (Gaddum, 1953a). Similar experiments with high concentrations of tryptamine were carried out on the rat's uterus, but the persistence of muscular contractions in the presence of tryptamine interfered with the results.

The phenomenon of tachyphylaxis was frequently observed on guinea-pig's ileum with tryptamine. The results of most of the experiments with this drug are described in conjunction with 5-hydroxytryptamine. Tryptamine was not very active on the guinea-pig's ileum and few experiments were done on this preparation.

Gramine. The effects of gramine are described on P.54-55. Gramine (0.5-50 mg/l.) inhibited the response of the guinea-pig's ileum to histamine sometimes less and sometimes more than the effects of 5-hydroxytryptamine and tryptamine. Frequently, it gave rise to small sustained contractions of the guinea-pig's ileum.

Cinobufotenin. It was about 2000 times less active than 5-hydroxytryptamine on the rat's uterus. High concentrations of the drug (6.5-50 mg/l.) caused marked/

Figure 29



Rat's uterus - 2 ml. bath

HT - 5-hydroxytryptamine 5 ng

AC - acetylcholine 200 ng

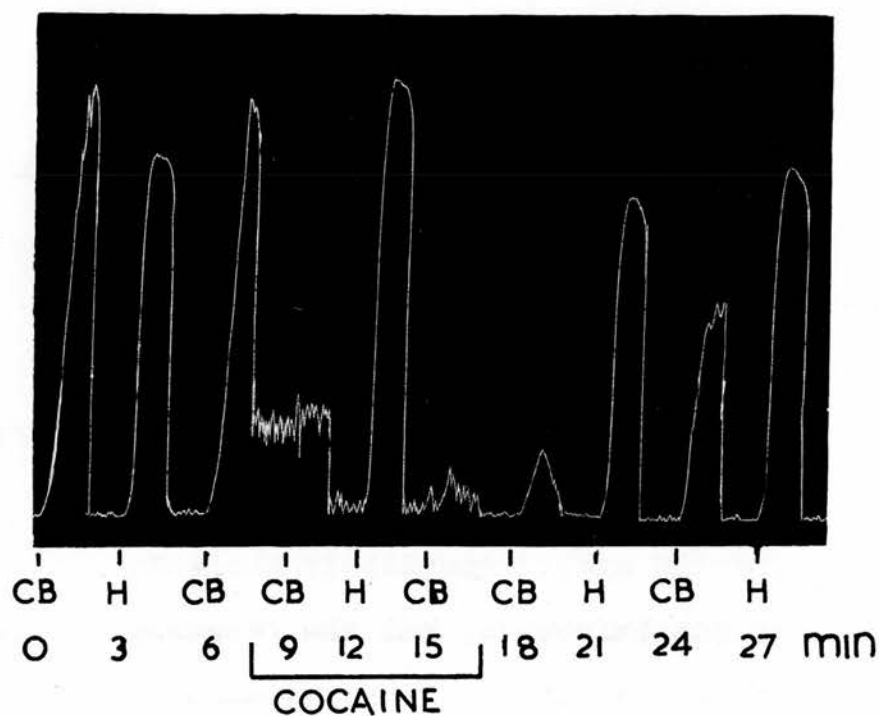
T - tryptamine 2000 ng

CB - cinobufotenin (6.5 mg/l.) for 10 min and washed out. The response to acetylcholine was abolished but responses to HT and tryptamine were unchanged.

marked contraction of the uterus. These concentrations were kept in the bath for 10 minutes and then washed out. The subsequent direct effects of 5-hydroxytryptamine and tryptamine were immediately increased above the normal level but the effect of choline ester was completely inhibited (Fig.29). Low concentrations (1.25-2.5 mg/l.) of the drug occasionally produced slight potentiation of the response to carbachol. Cinobufotenin is an inhibitor of cholinesterase (Sobotka and Antopol, 1937). The effects of cinobufotenin on the response to the choline ester may perhaps be explained on the same basis as those of eserine and ephedrine on the responses to acetylcholine and adrenaline. Low concentrations of eserine and ephedrine potentiate and higher concentrations of these drugs inhibit some of the actions of acetylcholine and adrenaline respectively.

As compared to the rat's uterus, cinobufotenin was 2000 times more active on the guinea-pig's ileum. It was 1/10 as active as 5-hydroxytryptamine on this preparation. In a concentration of 12.5 mg/l. it gave a sharp contraction of guinea-pig's ileum followed/

Figure 30



Guinea-pig's ileum - 2 ml. bath

CB - cinobufotenin 1500 ng

H - histamine 10 ng

Cocaine (10 mg/l.)  
abolished the response to CB  
but potentiated that to  
histamine.



followed by immediate relaxation. This concentration was then kept in the bath. The response to 5-hydroxytryptamine was completely blocked and the effect of histamine was partially inhibited.

Atropine in a concentration of 5  $\mu$ g/l. caused partial inhibition of the effect of cinobufotenin on guinea-pig's ileum (Fig.22). The effect of 5-hydroxytryptamine was unaffected, while the response to carbachol was completely abolished in the same experiment.

Hexamethonium in a concentration of 10 mg/l. produced partial inhibition of the response to cinobufotenin, but nicotine was completely blocked (Fig.27).

Cocaine (10 mg/l.) completely abolished the response to cinobufotenin, but the response to histamine was potentiated (Fig.30). Cocaine in this experiment, stimulated the guinea-pig's gut, (Feldberg and Lin, 1949).

High concentrations of 5-hydroxytryptamine (30 mg/l.) and tryptamine (50-100 mg/l.) desensitized the guinea-pig's ileum to 5-hydroxytryptamine and tryptamine but produced partial block of the response to cinobufotenin.

The above results seem to indicate that the action/

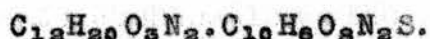
action of cinobufotenin on the guinea-pig's ileum consists of two components, the 5-hydroxytryptamine-like and the nicotine-like. The effect of the 5-hydroxytryptamine-like component was blocked by high concentrations of 5-hydroxytryptamine, but not affected by hexamethonium, while the reverse was true for the nicotine-like component. Cocaine completely inhibited the effect of cinobufotenin and cocaine is known to abolish the effects of both 5-hydroxytryptamine and nicotine.

If the theory (P.82) that 5-hydroxytryptamine and nicotine act on separate receptors or cells in the parasympathetic ganglia in the guinea-pig's ileum is accepted, then it seems most likely that cinobufotenin (the quaternary base corresponding to 5-hydroxytryptamine) acts on both these sites. The evidence for such an assumption is not yet conclusive.

The inhibition of the effect of cinobufotenin by a small dose of atropine (5  $\mu$ g/l.) is not fully understood. It may be explained on the assumption that the nicotinic effect is more easily suppressed by atropine than the 5-hydroxytryptamine effect.

Virido/

Viridobufotenin B. It was isolated from the parotid secretion of the green toad of Europe, *Bufo Viridis Viridis* (Chen, Jensen and Chen, 1933) in the form of a flavianate and gives positive reactions in indole tests. It has the following composition:



Viridobufotenin was found to have very little blood pressure raising power, having only 4 per cent of the pressor activity of cinobufotenin flavianate. It increases the tone of the frog's heart, stimulates isolated rabbit's intestine in the concentration of 1:10<sup>6</sup> and causes spastic contractions of the isolated guinea-pig's uterus in a solution of 1:200,000 (Chen, Jensen and Chen, 1933).

A very small sample of this drug was available and therefore its effects could not be properly studied.

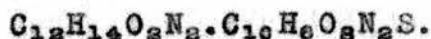
It stimulated the rat's uterus and was 20 times more active than cinobufotenin on this preparation and 100 times less active than 5-hydroxytryptamine. In a concentration of 100-2500 µg/l. it did not affect the responses of the rat's uterus to 5-hydroxytryptamine, tryptamine and carbachol.

It/

It also stimulated the guinea-pig's ileum and had about the same activity on this preparation as on the rat's uterus when compared with 5-hydroxy-tryptamine.

Cinobufotenin, however, was about 20 times more active than viridobufotenin on the guinea-pig's ileum. In concentrations of 2500 µg/l. it had no significant effect on the responses to 5-hydroxy-tryptamine and histamine.

Marinobufotenin. Chen, Jensen and Chen (1932) isolated marinobufotenin as the flavianate from the parotid secretion of the Jamaican toad or *Bufo marinus*. Its pharmacological actions were studied by Chen and Chen (1933b). It gives a positive indole reaction and has the following composition:



The physiological effects of marinobufotenin are much less prominent than those of cinobufotenin. In pithed cats, doses from 0.25 to 0.5 mg of the flavianate caused practically no increase in blood pressure. There was a slight decrease of the frog's heart rate and slight increase in its tone. A concentration of 1:200,000 brought about contractions of/

of the isolated guinea-pig's virgin uterus, but had no effect on the isolated rabbit's intestine.

In concentrations of 1.25-25 mg/l. it caused slight stimulation of the rat's uterus and had no effect on the responses to 5-hydroxytryptamine, tryptamine and carbachol.

Marino-bufotenin (25 mg/l.) did not cause any stimulation of the guinea-pig's ileum and had no effect on the contractions of 5-hydroxytryptamine and histamine.

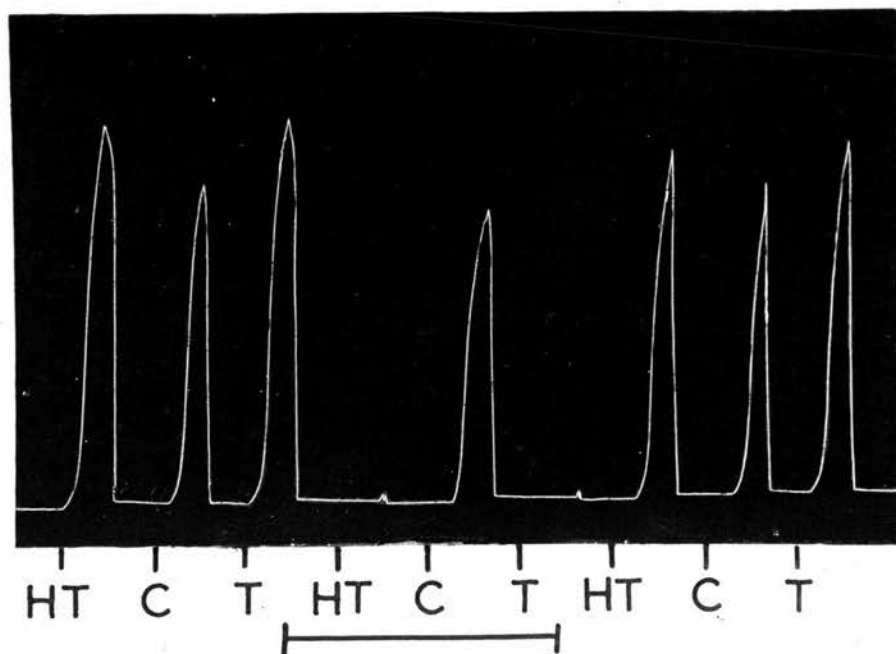
Indole. In concentrations of 5-25 mg/l. indole produced a feeble stimulant effect on the rat's uterus and inhibited the responses to tryptamine but the effect of carbachol was likewise inhibited.

On the guinea-pig's ileum, indole (0.5 - 5 mg/l.) occasionally produced excitation and caused slight inhibition of the responses to 5-hydroxytryptamine, tryptamine and histamine. In concentrations of 50 mg/l. it produced marked contraction followed by immediate relaxation, and the subsequent effects of 5-hydroxytryptamine, tryptamine and histamine were markedly inhibited.

2, 3 and 7-methyl indoles are insoluble and their saturated solutions were used.

2-methyl/

Figure 31



Rat's uterus - 2 ml. bath - interval 3 min.

HT - 5-hydroxytryptamine                      20 ng

C - carbachol                                      100 ng

T - tryptamine                                    4000 ng

2-methyl indole ( $\neq$  25 mg/l. during time  
signal) abolished the responses to HT  
and tryptamine without affecting the  
response to carbachol.

2-methyl indole in concentrations not greater than 25 mg/l. inhibited the responses of the rat's uterus to 5-hydroxytryptamine and tryptamine, but had no effect on the response to carbachol (Fig.31).

On the guinea-pig's ileum, 2-methyl indole in concentrations not greater than 50 mg/l. produced a non-specific inhibition of the effects of 5-hydroxytryptamine and tryptamine, as the response to histamine was also inhibited to a marked extent.

3-methyl indole (skatole) in concentrations not greater than 50 mg/l. inhibited the response of rat's uterus to tryptamine to a marked extent, but the response to carbachol was also inhibited though less than that to tryptamine.

On the guinea-pig's ileum, in the same concentrations as were used on the rat's uterus, it inhibited the responses to 5-hydroxytryptamine, tryptamine and histamine.

Skatole was less active and less specific as compared to 2-methyl indole. Both 2-methyl indole and skatole are also known as antihistamine agents (Bunyatyon and Matinyon, 1948).

7-methyl/



7-methyl indole in concentrations not greater than 50 mg/l. inhibited completely the effect of tryptamine on the rat's uterus and caused marked inhibition of the response of this preparation to carbachol.

On the guinea-pig's ileum in concentrations not greater than 25 mg/l. it sometimes produced stimulation and had no effect on the responses to 5-hydroxytryptamine, tryptamine and histamine.

3-indolyl-acetic acid (Heteroauxin). 3-indolyl-acetic acid (10 - 25 mg/l.) markedly inhibited the effects of 5-hydroxytryptamine, tryptamine and carbachol on the rat's uterus.

On the guinea-pig's ileum, 3-indolyl-acetic acid (0.5 - 50 mg./l.) produced a partial inhibition of the responses to 5-hydroxytryptamine, tryptamine and histamine. The response to histamine was slightly more inhibited than the response to 5-hydroxytryptamine or tryptamine. Occasionally, it produced a feeble stimulation of the guinea-pig's ileum.

Methyl-3-indolyl acetate (5 - 50 mg/l.) caused marked inhibition of the responses of rat's uterus to 5-hydroxytryptamine and tryptamine. The effect of a choline ester was also inhibited. Occasionally, it/

it produced contractions of the rat's uterus.

On the guinea-pig's ileum it was more active than the 3-indolyl-acetic acid. In concentrations of 25 - 50 mg/l. it inhibited the effect of 5-hydroxytryptamine to a marked extent. The response to histamine was also greatly suppressed. Like 3-indole-acetic acid, it occasionally caused stimulation of the guinea-pig's ileum.

Indole-propionic acid (25 mg/l.) had no effect on the responses of the rat's uterus to carbachol and tryptamine.

On the guinea-pig's ileum, it (5 - 50 mg/l.) produced a slight inhibition of the effect of 5-hydroxytryptamine, tryptamine and histamine.

Indole-butyric acid was insoluble and a saturated solution was prepared. It did not show much activity in the concentrations used.

In concentrations not greater than 25 mg/l. it had no effect on the responses of the rat's uterus. It caused a weak contraction of the rat's uterus.

Indole-butyric acid in concentrations not greater than 50 mg/l. had no effect on the actions of 5-hydroxytryptamine and tryptamine on the guinea-pig's ileum. The response to histamine was partially inhibited./

inhibited.

Isatin (2:3-indolinedione) was also inactive. It produced no significant effect on the responses of the rat's uterus and the guinea-pig's ileum to 5-hydroxytryptamine and tryptamine.

Isatin- $\beta$ -oxime was insoluble and a saturated solution was used. In a concentration not greater than 50 mg/l. it caused marked inhibition of the effects of 5-hydroxytryptamine and partial inhibition of the effect of tryptamine on the rat's uterus, without much effect on acetylcholine response.

On guinea-pig's ileum, isatin- $\beta$ -oxime was not very active. In concentrations not greater than 50 mg/l., it caused a partial inhibition of the responses to 5-hydroxytryptamine and tryptamine. The response to histamine was also partially blocked.

Tryptophan did not produce any significant effect on the responses of the rat's uterus and the guinea-pig's ileum to 5-hydroxytryptamine or tryptamine. The effects of choline esters and histamine were also not significantly affected. On guinea-pig's ileum concentrations of 50 mg/l. produced only partial inhibition of the effects of 5-hydroxytryptamine/

5-hydroxytryptamine and histamine.

Hypaphorine (tryptophan-betaine), in concentrations of 1.25 - 25 mg/l., caused spontaneous activity of the rat's uterus, but had no inhibitory effect on the responses to 5-hydroxytryptamine and tryptamine.

On the guinea-pig's ileum, hypaphorine (50mg/l.) caused no stimulant effect and had no action on the responses to 5-hydroxytryptamine and histamine.

Adrenochrome, was prepared by the action of silver oxide on adrenaline in methyl alcohol, (Braconier, Le Bihan and Beaudet, 1943). Its solution was made in ascorbic acid and tested when fresh.

In concentrations of 50  $\mu$ g/l. it markedly inhibited the effect of tryptamine on the rat's uterus. A concentration of 500  $\mu$ g/l. completely abolished the response to tryptamine. The effect of carbachol was likewise inhibited by the same concentrations.

Higher doses of adrenochrome (5-50 mg/l.) were required to abolish the effect of 5-hydroxytryptamine on the guinea-pig's ileum. The response to histamine was/

was also inhibited though less than that to 5-hydroxy-tryptamine.

It was considered that part of the effect of adrenochrome might be due to the presence of small quantities of active adrenaline in it. The effects of adrenochrome, therefore, were compared with those of a standard solution of adrenaline on the rat's uterus, and the rat's colon by the method depending on inhibition of the contractions of these tissues induced by carbachol (Gaddum, Peart and Vogt, 1949). The following results were obtained.

Rat's uterus:

1 ng of adrenaline = 300 ng of adrenochrome.

Rat's colon:

20 ng of adrenaline =  $> 10,000$  ng of adrenochrome.

$< 15,000$  ng of adrenochrome.

(ratio 500 to 750).

*for their actions on*  
When compared with the response to tryptamine on the rat's uterus, 0.5 ng adrenaline = 300 ng adrenochrome (ratio 600). The above results did not give a significant difference between the effects of adrenochrome and adrenaline on the two tissues tested. As the effect of tryptamine was also inhibited by very/

very low concentrations of adrenaline (1  $\mu\text{g}/\text{l.}$  or less), the presence of 0.17 % of adrenaline in the adrenochrome could not be ruled out. The concentrations of adrenochrome actually used to inhibit the response of the rat's uterus to tryptamine were 50 - 500  $\mu\text{g}/\text{l.}$  On the guinea-pig's ileum, still higher concentrations (5 -50  $\text{mg}/\text{l.}$ ) of adrenochrome were used to inhibit the effect of 5-hydroxytryptamine.

It did not produce any stimulation of the rat's uterus or the guinea-pig's ileum in the concentrations used.

Adrenolutine It was insoluble and very unstable. It was used in the form of a very fine emulsion. Adrenolutine (concentration not greater than 50  $\text{mg}/\text{l.}$ ) caused marked inhibition of the response of the rat's uterus to tryptamine. The effect of carbachol was also inhibited though not to the same extent as that of tryptamine. It did not stimulate the rat's uterus in the concentration used.

On the guinea-pig's ileum, adrenolutine (concentration not greater than 50  $\text{mg}/\text{l.}$ ) produced a small sustained contraction and partially inhibited the responses/

responses to both 5-hydroxytryptamine and histamine.

The results of the experiments with all the above indole compounds are listed in Table I and II.

This preliminary study revealed that all these indole compounds produced an unspecific inhibition of the effects of 5-hydroxytryptamine and tryptamine on the guinea-pig's ileum. In all cases, the effect of the control drug, histamine, was also inhibited although in most cases less than the effects of 5-hydroxytryptamine and tryptamine.

On the rat's uterus, gramine and 2-methyl-indole produced specific inhibition of the effects of 5-hydroxytryptamine and tryptamine in the sense that the response to a choline ester was unaffected by these drugs in the concentrations used. Isatin- $\beta$ -oxime also produced specific inhibition of the effects of 5-hydroxytryptamine and tryptamine but its action was feeble. All the other drugs caused an unspecific effect. Gramine was the most active antagonist for 5-hydroxytryptamine. It formed a prototype for the new synthetic compounds prepared by Messrs. Glaxo. Their actions and relative antagonistic activities are described in the next section.



Key to abbreviations used in TABLES I and II:

- ⊗ - Acetylcholine or carbachol.
- x - not tested
- 0 - no inhibition
- SI - slight inhibition (5-15%)
- PI - partial inhibition (16-50%)
- MI - marked inhibition ( $> 50\%$ )
- CI - complete inhibition
- S - saturated aqueous solution
- E - fine emulsion
- +
- ++ - contraction - relaxation, but spontaneous activity. Tested with agonists when spontaneous activity ceased.
- ‡ - occasional contraction
- # - no direct effect, spontaneous contractions set in after some minutes, which ceased after washing out. Agonists then added.
- \* - small sustained contraction.

Figure 32.

5-HYDROXYTRYPTAMINE

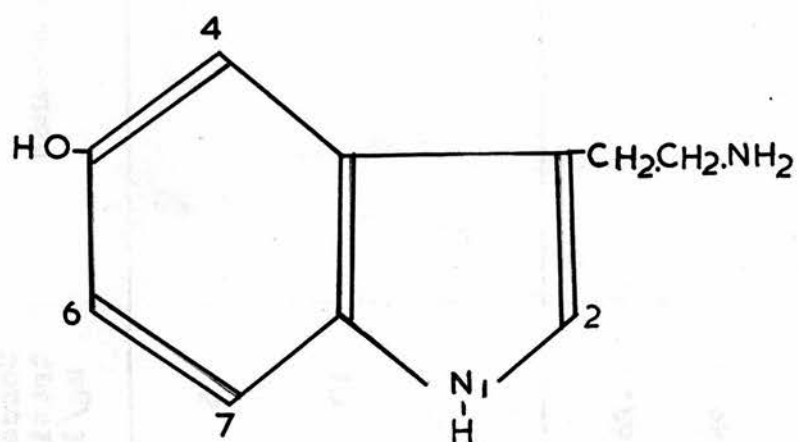
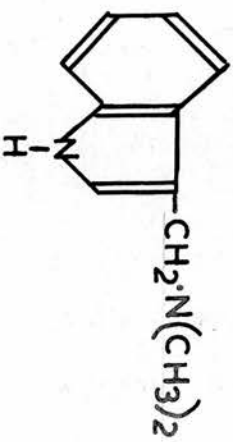
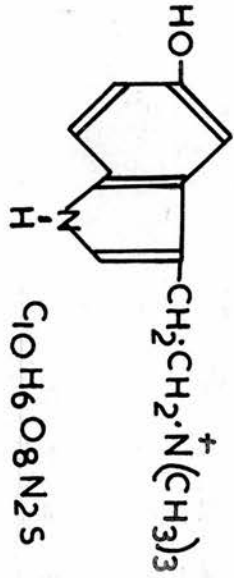


TABLE I

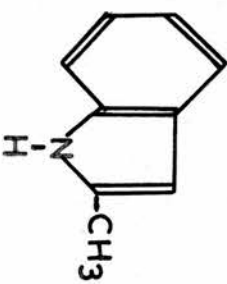
Effects of Indole Compounds on the sensitivity of the rat's uterus to 5-hydroxytryptamine, tryptamine and choline esters.

(HT = 5-10 µg/l.; Tryptamine = 0.5-15 mg/l.; Choline ester = 50-100 µg/l.)

Names of the Compounds	Concentration mg/l.	Action on the uterus	Inhibitory effect		
			HT (Fig. 32)	tryptamine	Choline ester
Gramine 	5	+	CI	CI	0
	10	+	CI	CI	0
Cinobufotenin flavianate 	0.25	+	0	0	0
	6.25	++	0	0	CI
	50	++	0	0	CI

⊗ Acetylcholine or carbachol.

TABLE I - Contd:

Names of Compounds	Concentration mg/l.	Action on the uterus	Inhibitory effect		
			HT	trypta- mine	Choline ester
Viridobufotenin flavianate $C_{18}H_{26}O_8Na \cdot C_{10}H_8O_8NaS.$	0.1 1.25 2.5	++ ++ ++	0 0 0	0 0 0	0 0 0
Marinobufotenin flavianate $C_{18}H_{14}O_8Na \cdot C_{10}H_8O_8NaS.$	1.25 6.25 25	- # #	0 0 0	0 0 0	0 0 0
Indole	5 25	+- +	x x	SI CI	SI MI
2-methyl indole <sup>S</sup> 	> 25	-	CI	CI	0

<sup>S</sup> Saturated aqueous soln. # No direct effect, spontaneous contractions set in after some mins. which ceased after washing out. Agonists then added.

TABLE I - Contd:

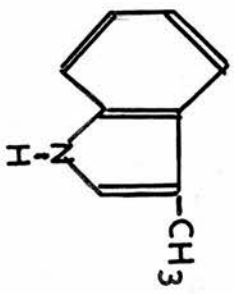
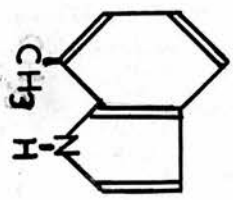
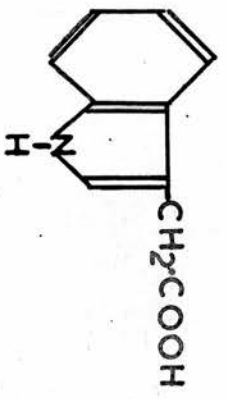
Names of Compounds	Concen- tration mg/l.	Action on the uterus	Inhibitory effect		
			HI	trypta- mine	Choline ester
3-methyl indole <sup>S</sup> 	$\neq$ 25 $\neq$ 50	-	x	MI	PI
			x	MI	MI
7-methyl indole <sup>S</sup> 	$\neq$ 50	-	x	CI	MI
3-indolyl-acetic acid 	10 25	-	PI	MI	MI
			MI	CI	CI

TABLE I - Contd:

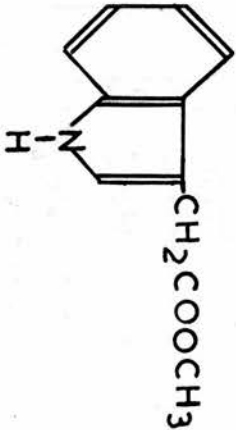
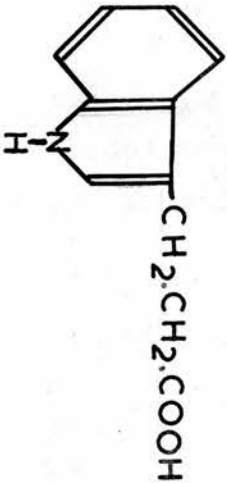
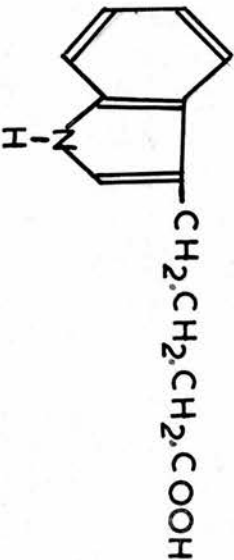
Names of Compounds	Concen- tration mg/l.	Action on the uterus	Inhibitory effect		
			HT	trypta- mine	Choline ester
Methyl-3-indolyl acetate 	5	±	0	PI	0
	25	±	x	CI	MI
	50	±	CI	CI	CI
Indole-propionic acid 	25	-	x	0	0
Indole-butyric acid <sup>S</sup> 	25	+	x	0	0

TABLE I - Contd:


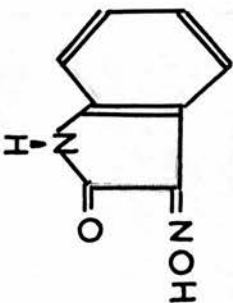
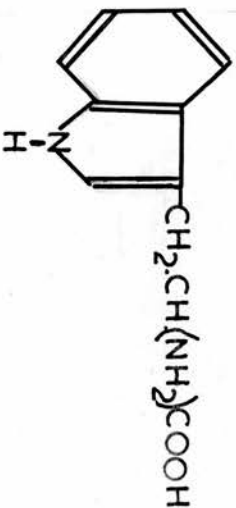
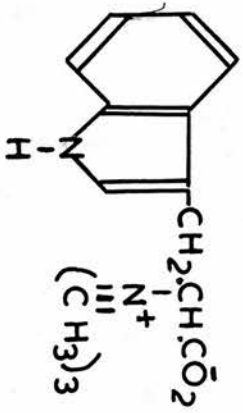
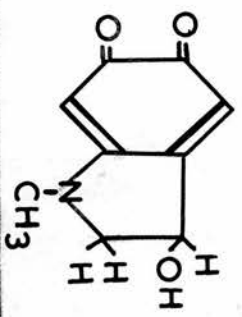
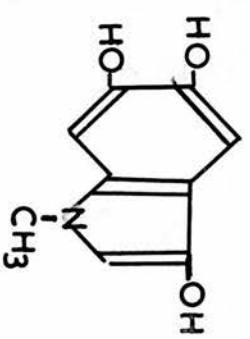
Names of the Compounds	Concentration mg/l.	Action on the uterus	Inhibitory effect		
			HF	trypta- mine	choline ester
2:3-Indolinedione (Isatin)	50	-	x	SI	0
					
Isatin- $\beta$ -oxime <sup>S</sup>	<del>25</del> 50	-	x MI	PI	0
					
dL-Tryptophan	25	-	x	0	0
					



TABLE I- Contd.

Names of the Compounds	Concen- tration mg/l.	Action on the uterus.	Inhibitory effect		
			HP	trypta- mine	choline ester
Hypaphorine Hydrochloride 	1.25	-	0	0	0
	6.25	#	0	0	0
	25	#	0	0	0
Adrenochrome 	0.05	-	x	MI	MI
	0.5	-	x	CI	CI
Adrenolutine <sup>E</sup> 	5	-	x	MI	SI
	50	=	x	CI	MI

<sup>E</sup>Fine emulsion. # No direct effect, spontaneous contractions set in after some mins. which ceased after washing out. Agonists then added.

TABLE II

Effects of Indole Compounds on the sensitivity of the

Guinea-pig's ileum to 5-hydroxytryptamine, tryptamine and histamine.

(HT = 50-100 µg/l.; Tryptamine = 15-30 mg/l.; Histamine = 5-10 µg/l.)

Names of the Compounds	Concentration mg/l.	Action on the ileum	Inhibitory effect		
			HT	tryptamine	Histamine
Gramine	0.5	±	x	PI	PI
	5	±	PI	x	MI
	50	±	CI	CI	CI
Cinobufotenin Flavanate	12.5	+	CI	x	PI

TABLE II - Contd:

Names of the Compounds	Concentration mg/l.	Action on the ileum	Inhibitory effect		
			HT	trypta- mine	Hista- mine
Viridobufotenin flavianate	2.5	+	O-PI	x	O-PI
$C_{12}H_{26}O_3N_2 \cdot C_{10}H_6O_8N_2S$ .					
Marinobufotenin flavianate					
$C_{12}H_{14}O_3N_2 \cdot C_{10}H_6O_8N_2S$ .	25	-	O	x	O
Indole					
	0.5	±	SI	SI	SI
	5	±	SI	x	SI
	50	+	MI	MI	MI
2-methyl indole <sup>s</sup>					
	25	-	MI	MI	MI
	50	-	CI	x	CI

TABLE II - Contd:

Names of the Compounds	Concen- tration mg/l.	Action on the ileum	Inhibitory effect		
			HT	trypta- mine	Hista- mine
3-methyl indole <sup>S</sup>	≠ 25	-	PI	PI	PI
	≠ 50	-	MI	x	MI
7-methyl indole <sup>S</sup>	≠ 25	±	0	0	0
3-Indolyl-acetic acid	0.5	±	x	SI	SI
	5	-	SI	x	PI
	50	-	PI	x	PI

TABLE II - Contd:

Names of the Compounds	Concentration mg/l.	Action on the ileum	Inhibitory effect		
			HT	trypta- mine	Hista- mine
Methyl-3-indolyl acetate	5	+	0	0	0
	25	-	MI	x	MI
Indole-propionic acid	5	±	SI	x	SI
	25	±	SI	SI	SI
	50	±	SI	x	SI
Indole-butyric acid <sup>s</sup>	25	±	0	0	SI
	50	±	0	x	PI

TABLE II - Contd:

Names of the Compounds	Concen- tration mg/l.	Action on the ileum	Inhibitory effect		
			HT	trypta- mine	Hista- mine
2:3-Indolinedione (Isatin)	5	-	0	x	0
	25	-	0	x	0
	50	+	SI	SI	SI
Isatin- $\beta$ -oxime <sup>S</sup>	$\neq$ 25	-	PI	PI	PI
	$\neq$ 50	-	PI	PI	PI
dl-Tryptophan	5	-	0	x	0
	25	-	0	x	0
	50	-	PI	x	PI

TABLE II - Contd:

Names of the Compounds	Concen- tration mg/l.	Action on the ileum	Inhibitory effect		
			HT	trypta- mine	Hista- mine
Hypaphorine Hydrochloride	0.5	-	0	x	0
	50	-	0	x	0
Adrenochrome	0.5	-	SI	x	0
	5	-	PI	x	PI
	50	-	CI	x	MI
Adrenolutine <sup>E</sup>	25	*	PI	x	PI
	50	*	PI	x	PI



Quantitative analysis of anti-5-hydroxytryptamine  
potencies of indole compounds.

A large series of indole and carbazole derivatives were tested in order to find a specific and potent anti-5-hydroxytryptamine agent. The anti-5-hydroxytryptamine potencies of various ergot alkaloids, dibenamine, atropine, mepyramine and diphenhydramine were also estimated. The antagonism of some of these drugs and 5-hydroxytryptamine have been studied by Erspamer (1954) and Shaw and Woolley (1953).

Experiments in which antagonistic drugs are compared with one another are complicated by the fact that there is no generally accepted way of measuring their potencies. Rough measurements may be made by finding the dose necessary to abolish the effect of the active drug ("the agonist") completely. Such measurements are unlikely to be very accurate since the result will depend on the dose of agonist used, and on the sensitivity of the record to small effects produced in the presence of the antagonist. A few preliminary experiments of this type were performed and confirmed the impression that it was not very reliable.

reliable.

It is probably more satisfactory to use a method depending in some way on a measurement of the effect produced by the antagonist.

When the effect of a given dose of agonist is inhibited completely, it is generally found that larger doses are still effective, but can be blocked by larger doses of the antagonist. Such results are sometimes described by calling the antagonism competitive (Shaw and Woolley, 1953).

This practice may lead to confusion since it suggests that it has been proved that the two drugs are competing with one another for the same receptor in the tissue. It has however, been pointed out (Gaddum, 1943) that studies of the quantitative relations between the concentrations of antagonists needed for a constant effect provide no evidence to distinguish the theory that the drugs are competing with one another for the same receptor, and the theory that the drugs are combining with one another to form an inactive complex. The expression "competitive antagonism" has been used for so long to describe the first of these theories, that it would be difficult to alter its meaning now. Some other word/

word therefore, needed to describe the actual observation that over a certain range of doses an increase of the dose of either drug overcomes the effect of its antagonist. It is proposed to call this type of antagonism surmountable.

The measurement of the effect of the antagonist.

The effect of the antagonist may be measured in terms of the percentage reduction of the recorded response to the agonist. This is only possible in a limited range of doses of the antagonist and is unlikely to give constant results. When experiments are made on isolated organs, measurements are therefore generally made of the following quantities.

$A_0$  - the concentration of the agonist which has a given effect ( $y$ ) in the absence of the antagonist.

$A$  - the concentration of the agonist which has the same effect in the presence of the antagonist in concentration  $B$  and at time  $t$ .

The dose ratio ( $A/A_0$ ) when the antagonism is surmountable is often taken as a measurement of the effect produced by the antagonist. Sometimes it is independent of  $y$ , so that the log dose effect curves before and after the antagonist are parallel. This is/

is however, not always so (Schild, 1949).

Cases are known in which  $A/A_0$  increases with  $y$ , so that the antagonist makes the log dose effect curve flatter, and cases are also known where the reverse is true. The quantity  $A/A_0$  can then only be used as an expression of the effect of the antagonist if  $y$  is kept constant and Schild (1949) has proposed that it should be 50 per cent of the maximum effect.

Rocha e Silva and his colleagues (Rocha e Silva and Beraldo, 1948; Beraldo and Rocha e Silva, 1949; Rocha e Silva, 1950) have suggested that the effect of an antagonist should be measured in terms of the time the muscle takes to recover when the antagonist is removed from the bath. They have studied this recovery in some detail and use more than one way of expressing their results, but the simplest one is  $R_{50}$  which is the time in seconds for the muscle to recover sufficiently to give a contraction half as great as the original contractions. This time increases with the dose and can be used as a measure of the effect of the antagonist. There is no evidence that the use of this quantity avoids the complications/

complications described above due to the fact that  $A/A_0$  is not independent of  $y$ . Rocha e Silva et al. avoid such complications by using a constant dose of the agonist.

The importance of this kind of measurement is emphasized by the work of Fleckenstein (1952) who found that the actions of specific antagonists often lasted longer than those of less specific drugs. On the other hand, duration of the action is not the only property of drugs which may be worth measuring.

Mepyramine antagonises the action of histamine on the guinea-pig's ileum in lower doses than promethazine but its action does not last so long (Reuse, 1948). If the meta meter proposed by Rocha e Silva et al. were used as the only criterion of the action of the antagonists, such differences would be obscured. In any case, some drugs have such a prolonged action that the use of this method of investigation would be impossible (Gaddum, 1926).

For these reasons, the ratio  $A/A_0$  is probably a more generally useful measure of the effect produced by an antagonist .

The/

The measurement of the potency of antagonists.

The potencies of antagonists may be compared by measuring, for each, the concentration (B) needed to have a standard effect ( $A/A_0$ ), in a standard time. This is the basis for the calculation of the  $pA_x$ , which is the negative logarithm of the molar concentration where  $A/A_0$  is  $x$  (Schild, 1947). This quantity only gives the potency in arbitrarily restricted conditions, but it has been found to give surprisingly constant results in different laboratories (Reuse, 1948). The concentration of an agonist needed to have a standard effect may vary enormously, but the concentration of an antagonist for a standard effect seems to vary much less.

The  $pA_2$  provides a measurement of the threshold concentration which just produces a small effect. The  $pA_{10}$  can generally be estimated more accurately and the conditions are such as to ensure that the antagonist is fairly potent. To estimate the  $pA_x$  it is necessary to expose pieces of tissue to various concentrations of the antagonist and to estimate in each case whether  $x$  is greater or less than the required/



required value. Schild (1947) estimated the  $pA_2$  by first getting a constant effect with a constant dose of agonist alone and then combining double this dose with a suitable concentration of the antagonist, and observing whether the effect produced at the standard time was greater or less than the original effect. The concentration for equal effects was then estimated by interpolation. This is probably the most satisfactory method of making accurate comparisons between antagonists, but may be a laborious way of studying the effects of a long series of drugs whose properties are at first quite unknown and whose effects may increase almost indefinitely with the time of exposure. Another method of estimating the potency of antagonists is described later.

#### Methods.

Rat's uterus in oestrous was found by Erspamer (1952b) to be particularly sensitive to 5-hydroxytryptamine and this tissue has been used in the experiments described here. The technique was the same as that described on P.40. The small bath/



bath was connected to two reservoirs, one containing the ordinary de Jalon's solution and the other, an appropriate concentration of the antagonist. When the effect of an antagonist had to be studied, the bath was filled from the reservoir containing the antagonist.

This preparation was chosen for this work mainly because simple indole compounds such as gramine were found to depress the response to 5-hydroxytryptamine in concentrations which did not depress the response to other drugs. When tested on other tissues such as the guinea-pig's ileum, the effect of gramine appeared to be less specific, and when sufficient concentrations were used to depress the response to 5-hydroxytryptamine, the response to other drugs was also depressed. In the experiments described here, acetylcholine was applied as a control drug in doses causing similar sub-maximal effects to those due to 5-hydroxytryptamine. The statement that the effect was specific means that the response to 5-hydroxytryptamine was depressed by doses of the antagonist which did not affect the response to acetylcholine.

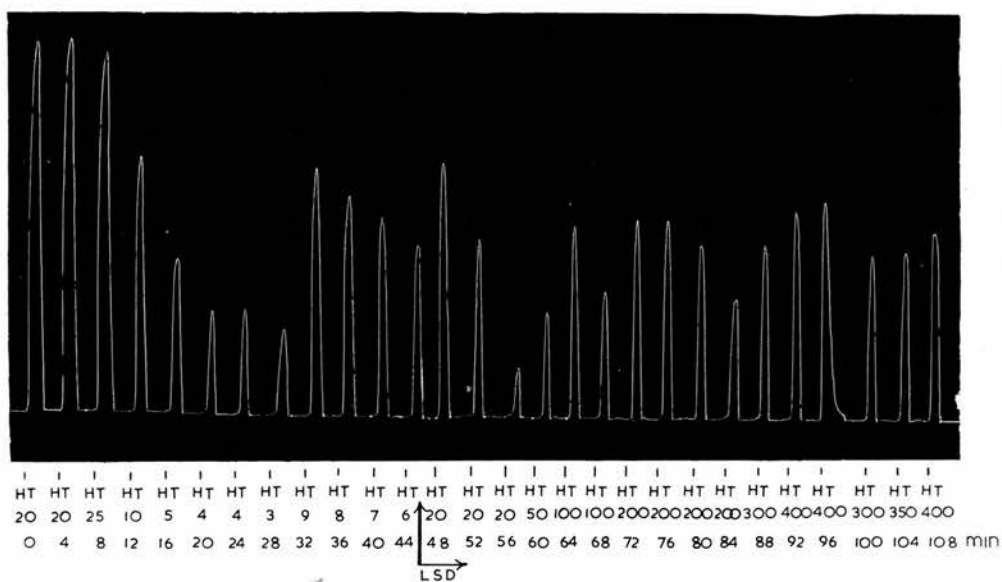
The preparations which gave constant responses to/

5-hydroxytryptamine were used for these experiments. A separate piece of a uterine horn was used for each experiment to avoid errors due to the persistence of antagonistic effects. All comparisons between the various drugs were made on separate pieces of the uterus.

### Results.

A dose of 10 - 15 ng of 5-hydroxytryptamine (calculated as base) generally caused a suitable contraction when added to the bath (2 ml). Some such dose was added at regular intervals (3-5min.) until a regular response was obtained. The dose was then altered and a curve constructed by plotting the height of the response against the dose. The drugs to be tested as antagonists were then added in an initial concentration of  $10^{-6}$  (1  $\mu\text{g}/\text{ml}$ ) and 5-hydroxytryptamine was reapplied. The concentration of the antagonist was maintained in the bath for 60 minutes, either by adding fresh doses whenever the fluid was changed or by adding the drug to one of the reservoirs from which the bath could be refilled. If the effect of 5-hydroxytryptamine was not definitely diminished, higher concentrations of the potential/

Figure 33



Rat's uterus - 2 ml. bath

Measurement of the dose ratio ( $A/A_0$ )

HT - 5-hydroxytryptamine - doses in ng

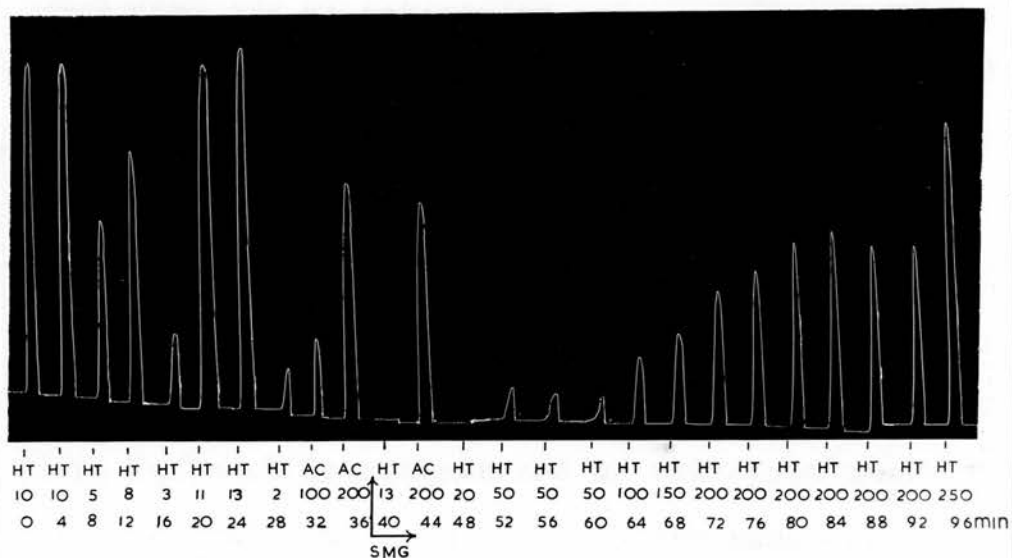
LSD - lysergic acid diethylamide (10  $\mu$ g/l.)  
added to de Jalon's solution at 45 min.

potential antagonists were tried. When the effect of 5-hydroxytryptamine was diminished, the dose of 5-hydroxytryptamine was increased until the response was 50 per cent of the maximum response at the beginning of the experiment, or until it became apparent that this result could not be achieved.

Time relations and dose effect curves.

The effects of active and specific antagonists were calculated in terms of the dose ratio ( $A/A_0$ ) by comparing effects in the presence of the antagonist with the initial dose effect curve. Fig.33 shows an experiment of this kind with lysergic acid diethylamide. After the dose effect curve of 5-hydroxytryptamine was plotted, lysergic acid diethylamide in a concentration of 10  $\mu\text{g}/\text{l}$ . was added to the bath at 45 minutes and this concentration was maintained in the bath for 60 minutes or more. After 3 minutes contact with lysergic acid diethylamide, a dose of 20 ng of 5-hydroxytryptamine produced an effect equal to that caused by 10 ng of 5-hydroxytryptamine at the beginning of the experiment. The dose ratio ( $A/A_0$ ), therefore, was 20/10 after 3 minutes. After 15 minutes, a dose of 50 ng of 5-hydroxytryptamine/

Figure 34



Rat's uterus - 2 ml. bath

Measurement of the dose ratio ( $A/A_0$ )

HT - 5-hydroxytryptamine - doses in ng

5-MG- 5-methylgramine (1 mg/l.) added to  
de Jalon's solution at 37 min.

121.

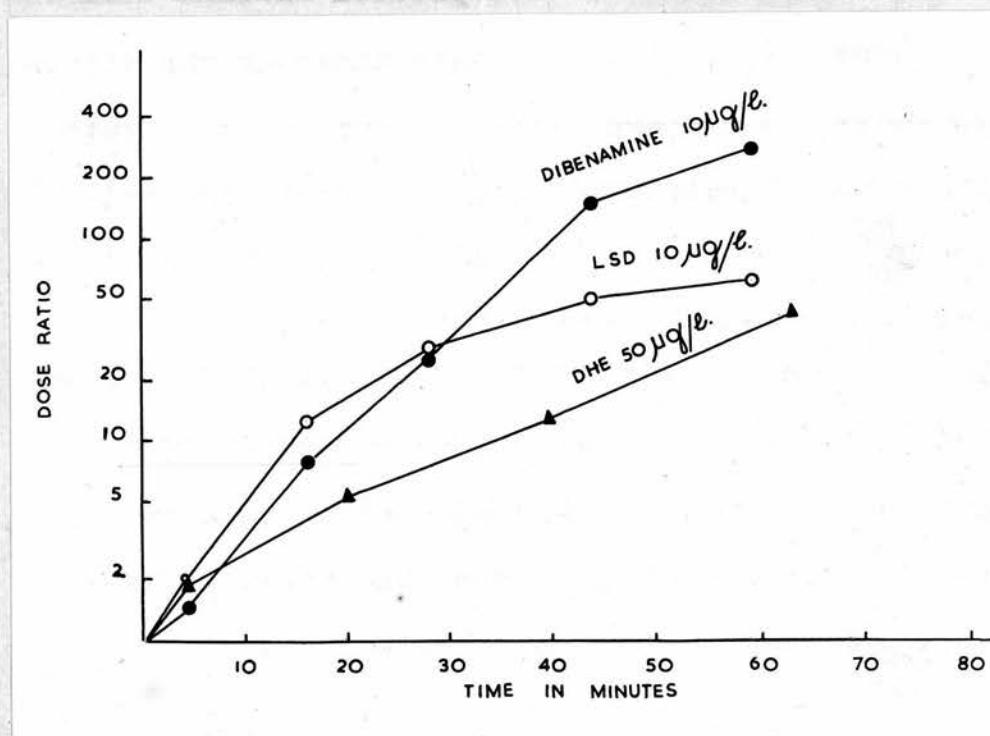
5-hydroxytryptamine produced an effect similar to that caused by 4 ng of 5-hydroxytryptamine in the absence of lysergic acid diethylamide and the dose ratio became 50/4. This ratio went on increasing with time and after 27 minutes it was 200/7, after 43 minutes, 300/6 and after 59 minutes, 350/6.

A similar experiment with 5-methyl gramine is illustrated in Fig. 34. A concentration of 1 mg/l. of 5-methyl gramine was maintained in the bath from 37 minutes for an hour. Immediately, the responses to 13 and 20 ng of 5-hydroxytryptamine were completely suppressed, but after 15 minutes of the exposure to the antagonist, when the dose of 5-hydroxytryptamine was increased to 50 ng it caused an effect equal to about that caused initially by 2 ng of 5-hydroxytryptamine in the absence of 5-methyl gramine. The dose ratio was 50/2. It became 100/3 after 27 minutes and 200/5 after 43 minutes. After about 40 minutes of the addition of 5-methyl gramine to the de Jalon's solution, the dose ratio did not increase and remained more or less at the level of 40.




The equiactive doses of 5-hydroxytryptamine before/



**Figure 35**



Relation between dose ratio ( $A/A_0$  in log) and time

Dibenamine	
LSD - lysergic acid diethylamide	
DHE - dihydroergotamine	



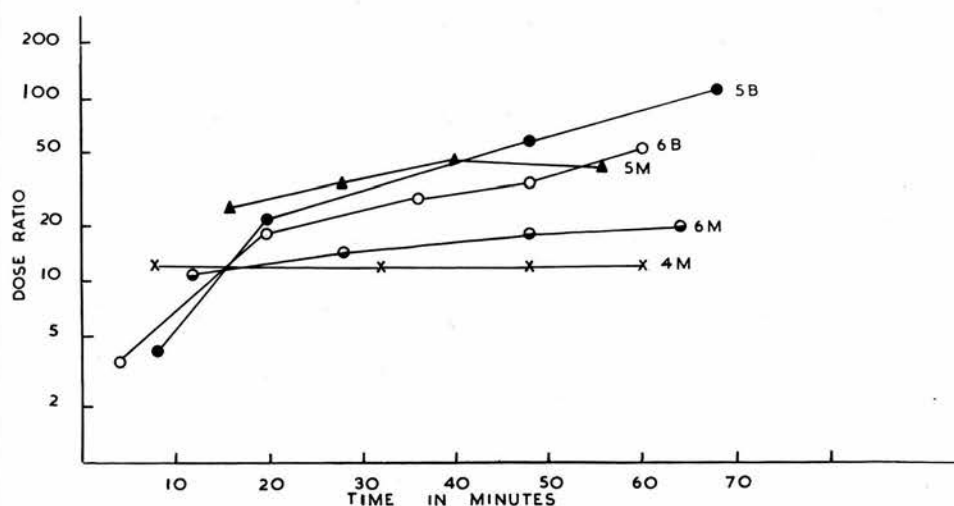
before and after the antagonist were carefully selected. The ordinary dose effect curve of 5-hydroxytryptamine was plotted in every case and if a dose corresponding to a particular height of the response was not available in the original series, it was read from the smooth curve constructed.

These ratios were then plotted against time and the curves for various concentrations of the different compounds compared (Fig. 35 and 36 ). Such curves were constructed only for those compounds which gave a dose ratio of 10, or higher, and had a specific anti-5-hydroxytryptamine action. The shapes of the curves obtained in this way may vary considerably. The three compounds shown in Fig. 35 were the most active compounds tested when judged by their effects after 1 hour, but these effects developed very slowly and after 10 minutes these compounds showed very little effect in the low concentrations used in these experiments. The effect of dihydroergotamine was particularly slow to develop.

Fig. 36 shows similar curves for 5 derivatives of gramine all used in the same concentration ( $10^{-6}$ ).

Most/

**Figure 36**



Relation between dose ratio ( $A/A_0$  in log) and time

Concn. 1 mg/l.

5B - 5-benzyloxy gramine

● ——— ●

6B - 6-benzyloxy gramine

○ ——— ○

5M - 5-methyl gramine

▲ ——— ▲

6M - 6-methyl gramine

○ ——— ○

4M - 4-methyl gramine

x ——— x

Most of these effects were also slow to develop.

The effects of most of the compounds were complete within 60 minutes in the concentrations used in the experiments. Sometimes, with <sup>the</sup> most active compounds even after 60 minutes it was impossible to be sure that the effect was complete, but long times introduce errors of their own and 60 minutes was adopted as the standard time at which measurements were made.

In low concentrations (10  $\mu\text{g}/\text{l.}$ ) the effects of lysergic acid diethylamide, dibenamine and dihydroergotamine showed more or less a state of equilibrium after 60 minutes but with higher concentrations of these drugs, the effects went on increasing indefinitely. The effects of dihydroergotamine in a concentration of 50  $\mu\text{g}/\text{l.}$  as shown in Fig. 35 was still increasing even after 60 minutes. In a concentration of 10  $\mu\text{g}/\text{l.}$  dihydroergotamine did not give a high dose ratio, but lysergic acid diethylamide and dibenamine were very active at this dose level.

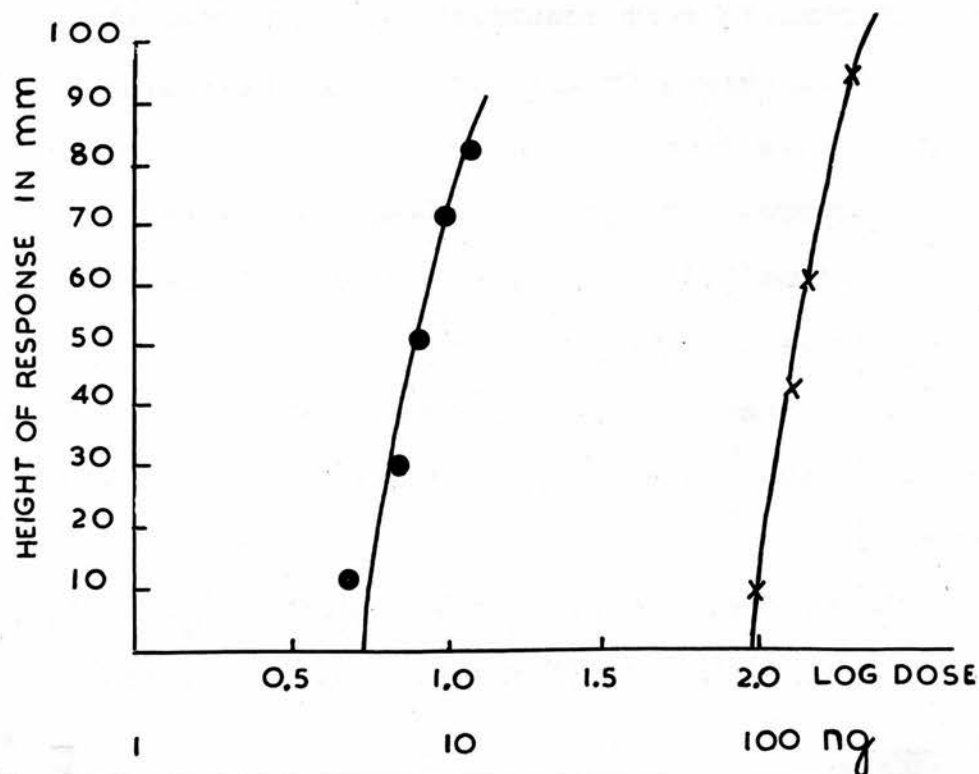
Dihydroergocornine in the concentration used (20  $\mu\text{g}/\text{l.}$ ) to give a specific effect caused a maximum dose/

dose ratio of 8.3 after 60 minutes. The effect continued to increase with this dose even after 1 hour. Higher concentration (50  $\mu\text{g}/\text{l.}$ ) of this compound produced an unspecific effect. Dihydroergokryptin was not very active. Even in a concentration of 100  $\mu\text{g}/\text{l.}$  the dose ratio increased only to 9.0 after 60 minutes. Its effect in this concentration was also progressive. In higher concentrations, it sometimes produced contractions and spontaneous activity. The log dose effect curves of 5-hydroxytryptamine in the presence of these antagonists were not therefore, properly studied.

The effect of 5-benzyloxy gramine was more progressive than that of 6-benzyloxy gramine. The effects of both these compounds were complete after about an hour. The dose effect curves of 5-hydroxytryptamine in the presence of the two benzyloxy gramines were also not properly studied as it took a long time for their effects to be completed.

The effects of methyl gramines were complete earlier (Fig.36 ). With 1, 5 and 6 methyl gramines, the state of equilibrium was established after 30-40 minutes. The effect of 4-methyl gramine, however, was/

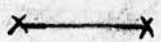
Figure 37



Log dose-effect curves of 5-hydroxytryptamine



Normal curve



Curve in the presence  
of 6-methyl gramine,  
(1 mg/l.)

was complete within 10 minutes. 1-methyl gramine did not show much activity in the concentration used (1 mg/l.). The dose ratio with this compound rose to the maximum figure of 6.6. The log dose effect curves of 5-hydroxytryptamine in the presence of methyl gramines were identical in slope with the log dose effect curves in the absence of these drugs. Fig.<sup>37</sup>. shows that the log dose effect curve of 5-hydroxytryptamine in the presence of 6-methyl gramine (1 mg/l.) is similar in slope to the log dose effect curve of 5-hydroxytryptamine in the absence of this compound. The dose ratio ( $A/A_0$ ) of methyl gramines, therefore, seemed to be independent of the effect  $y$ .

The four derivatives of tryptamine; 2-methyl tryptamine, N.N.dimethyl tryptamine,  $\alpha$ - $\alpha$ -dimethyl tryptamine and  $\alpha$ -ethyl tryptamine also showed specific but feeble anti-5-hydroxytryptamine effects. Their dose ratios did not increase to more than 10 in the ordinary concentrations used (1-10 mg/l.). N.N.-dimethyl tryptamine which was the most active from this series, gave a dose ratio of 10. The effects of all these tryptamine derivatives were complete in about half- /

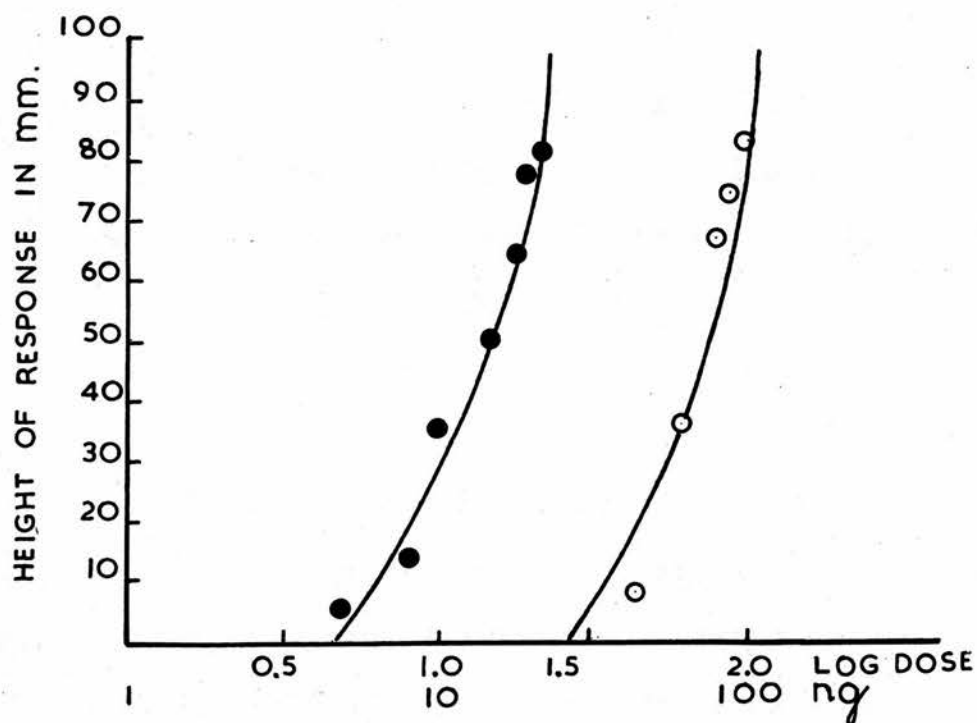
half-an-hour. The log dose effect curves of 5-hydroxytryptamine in the presence of these compounds were similar in slope to the original log dose effect curves of 5-hydroxytryptamine. Fig.38 shows that log dose effect curves of 5-hydroxytryptamine in the presence and absence of 2-methyl tryptamine are similar in shape.

The equilibrium state was established within 10 minutes with 5-amino-3-ethyl-2-methyl indole. The shape of the log dose effect curve of 5-hydroxytryptamine in its presence was also similar to the shape of the log dose effect curve in the absence of this compound (Fig.39).

This compound was synthesised by Woolley and Shaw (1952) and reported as a potent anti-5-hydroxytryptamine agent. It did not show much activity with the test used in this study (Table III ) in the concentrations employed (5 mg/l.). Erspamer and Ottolenghi (1953) also reported that the anti-enteramine activity of this compound was weak. However, Woolley and Shaw employed a different kind of test. They tested it on a ring-shaped segment of sheep carotid artery. A maximal contraction ( $1/3$  decrease in diameter), of the artery ring was produced/



Figure 38



Log dose-effect curves of 5-hydroxytryptamine

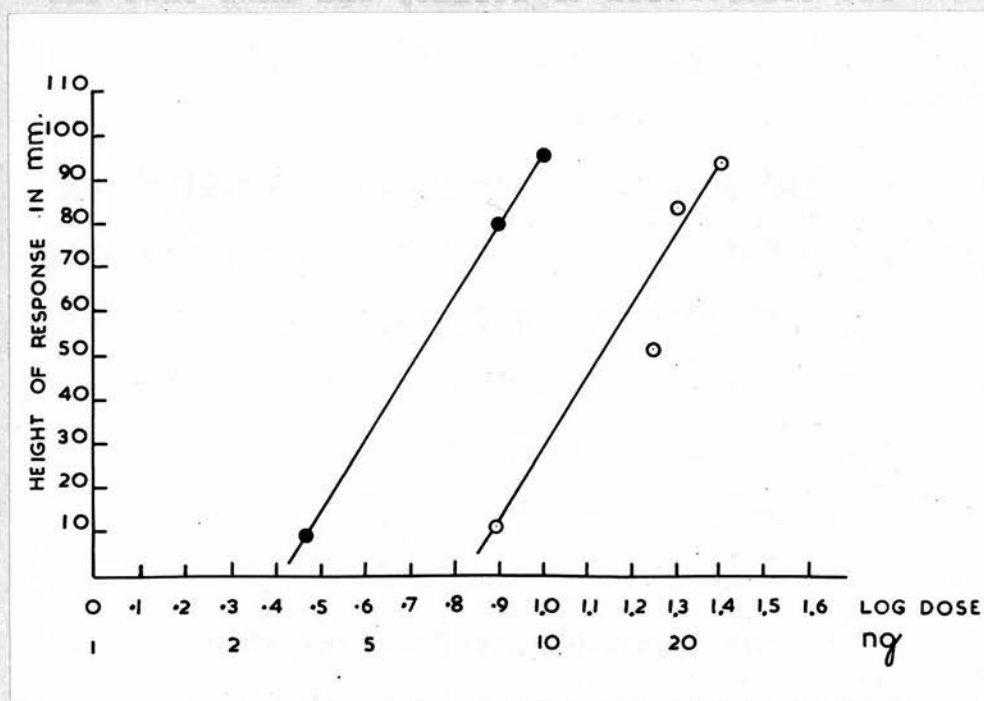


Normal curve

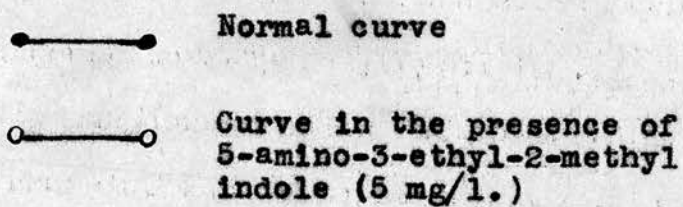


Curve in the presence of  
2-methyl-tryptamine,  
(10 mg/l.)

**Figure 39**



**Log dose-effect curves of 5-hydroxytryptamine**



produced by 0.2  $\mu$ g per ml of 5-hydroxytryptamine. Half maximal antagonism was observed with 5-10  $\mu$ g of this analog. This compound proved a failure in clinical trials (Spies and Stone, 1952; Iverson and Bull, 1953; Page and McCubbin, 1953b).

The observation of Woolley and Shaw that the corresponding nitro compound (Table IV) was inactive in vitro was also confirmed by tests employed in the present study, in the ordinary concentrations as used for other compounds but the experiments with this compound were unsatisfactory because it was so insoluble. The effect with 6-amino-2:3-dimethyl indole continued to increase for an hour or so in the concentrations used (5 mg/l.)

6-amino-tetrahydro carbazole was the only compound from the carbazole derivatives which showed a specific effect but was feeble in its action. The effect of this compound in the concentration used (5 mg/l.) was complete within 10 minutes and the pattern of the log dose effect curves of 5-hydroxytryptamine with this compound was the same as that described above.

When the experiments were repeated, the dose ratio/

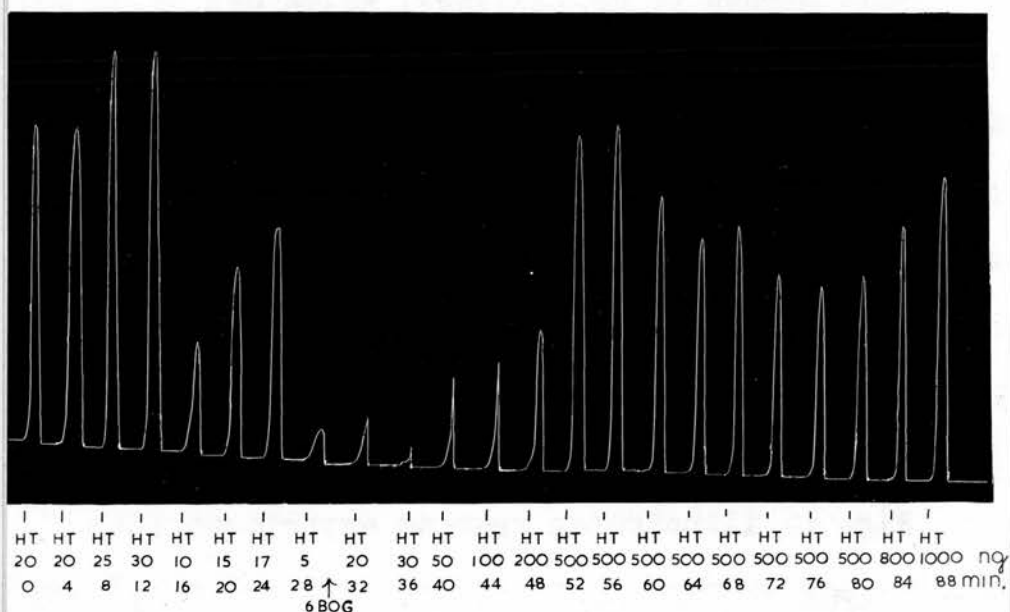
ratio in most cases gave constant results and only in few instances the results were variable. Such experiments were repeated with few compounds.

The experiments with time relation showed that with most active drugs the block developed gradually and went on increasing indefinitely while with less active compounds the effects were complete within 30 minutes.

The drug ratio.

The drugs can be compared with one another by measuring the effect produced after 60 minutes. This method can however, only be used when a concentration is found in which all the drugs have some effect. Some of the drugs in the present series had no effect at all in a concentration of  $10^{-6}$  but were effective in higher concentrations; others were effective in lower concentrations. The opinion has already been expressed that the most satisfactory general index of the activity of antagonists is the  $pA_x$ , but quicker results may be obtained by estimating the drug ratio, which is equal to the ratio of the concentration of the agonist to the concentration of the antagonist when the response is/

Figure 40



Rat's uterus - 2 ml. bath

Measurement of drug ratio (A/B)

HT - 5-hydroxytryptamine

6-BOG - 6-benzyloxy gramine (1 mg/l.) added to de Jalon's solution at 29 min.



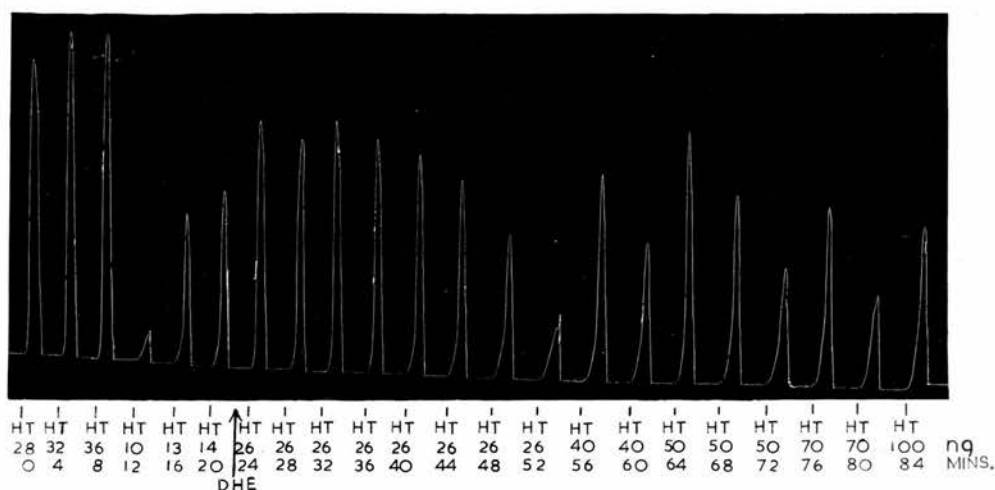
is 50 per cent of the initial maximum response.

Estimates of this quantity are unlikely to give reliable results when the dose ratio ( $A/A_0$ ) is small and the concentrations were therefore always high enough to produce a dose ratio of at least 5 and generally much more.

This method of calculation, which has often been used before, is convenient because it can be used to compare results when different concentrations of antagonists are used, as is necessary when the activities vary widely. It is more convenient than the  $PA_2$  in preliminary screening tests because an estimate can often be obtained in a single experiment using only one concentration of the antagonist.

Fig. 40 illustrates such an experiment with 6-benzyloxy gramine. A dose of 30 ng of 5-hydroxytryptamine produced a maximum effect on the rat's uterus. 6-benzyloxy gramine (1 mg/l.) was then continuously maintained in the bath from 29 minutes. It caused a marked decrease in the effect of 5-hydroxytryptamine, and the activity continued to increase. Higher doses of 5-hydroxytryptamine were applied at intervals to overcome this block and to get/

Figure 41



Rat's uterus - 2 ml. bath

Measurement of drug ratio (A/B)

DHE - dihydroergotamine (10  $\mu$ g/l.)  
added to de Jalon's solution  
at 21 min.



get a response which was 50 per cent of the initial maximum effect. The height of the response was kept more or less at the level of 50 per cent of the initial maximum response and the doses of 5-hydroxytryptamine which would give this height of response were always aimed at. After 51 minutes (corresponding to 80 min. in Fig. 40) a dose of 500 ng of 5-hydroxytryptamine in a 2 ml bath produced a response which was equal to 50 per cent of the initial maximum effect. The drug ratio, therefore, was  $250/1000 = (1/4)$ .

A similar experiment with dihydroergotamine is shown in Fig. 41. A dose of 100 ng of 5-hydroxytryptamine in a 2 ml bath in the presence of dihydroergotamine ( $10\mu\text{g}/\text{l.}$ ) after 63 minutes gave a response which was 50 per cent of the initial maximum response. The drug ratio in this case was  $50/10 = (5)$ .

The results shown in Tables III and IV were obtained in this way. The drugs listed in Table III had a specific antagonistic effect to 5-hydroxytryptamine and the compounds collected in Table IV had an unspecific effect as these drugs depressed the response to acetylcholine. Most of these drugs decreased the response to 5-hydroxytryptamine more than/

than acetylcholine and a few compounds suppressed the effect of acetylcholine as much as, or more than, the response to 5-hydroxytryptamine.

When the drug ratio is less than one it is given as a fraction in the belief that this is easier to remember than a decimal. The compounds in Table III are arranged according to their potencies and chemical structure but compounds in Table IV are grouped mainly according to their chemical constitution.

Most of the compounds provided for testing were very insoluble and it was found possible to dissolve them in a small volume of ethyl alcohol. Further dilutions were made up with de Jalon's solution and almost in all cases the sample remained in solution by this method but where it precipitated it was redissolved by the addition of more alcohol. The final concentration of alcohol in the bath was determined in all these cases. The inhibitory effects of different concentrations of alcohol on the response of rat's uterus to 5-hydroxytryptamine were found. Alcohol in concentrations greater than 0.25 per cent in the bath inhibited the response of the rat's uterus to 5-hydroxytryptamine. When the test solution contained alcohol in these concentrations, the antagonism was partly ascribed to alcohol.

TABLE III.

Comparative potencies of  
specific anti-5-hydroxytryptamine compounds.

Names of the compounds	Drug ratio  ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) 1 hour	Solubility
Dibenamine hydrochloride	35-50	Insoluble, dissolved in acidic normal saline to make a solution of $10^{-3}$ . Further dilutions made with de Jalon's solution and the drug remained in solution.
Lysergic acid diethyl amide (LSD)	20	
Dihydroergotamine methanesulphonate	4-5	
Dihydroergocornine methanesulphonate	1.25	Soluble in warm acidic normal saline solution.
Dihydroergokryptine methanesulphonate	1/2-1/1.3	Soluble in warm acidic normal saline solution.

Table III Contd:

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) 1 hour	Solubility
5-benzyloxy gramine	1/2.4	Dissolved in warm acidic normal saline solution.
6-benzyloxy gramine	1/4	Dissolved in warm acidic normal saline solution.
5-methyl gramine	1/10	Soluble in acidic normal saline solution.
6-methyl gramine	1/16	Soluble in acidic normal saline solution.
4-methyl gramine	1/28	Soluble in acidic normal saline solution.
1-methyl gramine	1/80	Light brown liquid easily soluble in normal saline solution.
N:N:dimethyltryptamine	1/40	Soluble in acidic normal saline solution.
$\alpha$ - $\alpha$ -dimethyl tryptamine	1/72	Easily soluble in normal saline solution.
$\alpha$ -ethyl tryptamine	1/200	Easily soluble in normal saline solution.

Table III Contd:

Names of the compounds	Drug ratio concentration of HT (concentration of antagonist) 1 hour	Solubility
2-methyl tryptamine	1/400	Easily soluble in normal saline solution.
6-amino-2:3:dimethyl indole	1/84	Soluble in warm acidic normal saline solution.
5-amino-3-ethyl-2-methyl indole	1/660	Easily soluble in normal saline solution.
6-aminotetrahydro-carbazole	1/220	Easily soluble in acidic normal solution.

5-hydroxy indole, 6-hydroxy indole and tryptophol had very little or no effect, on the response to 5-hydroxytryptamine in the concentrations used (10-100 mg/l.). The response to acetylcholine was also not affected by these drugs.

TABLE IV.

Comparative potencies of  
unspecific anti-5-hydroxytryptamine compounds.

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ 1 hour	Solubility	Remarks
1-methyl indole	1/250	Yellow liquid, soluble only in alcohol.	
4-methyl indole	1/660	Greenish watery liquid, soluble in alcohol.	
5-methyl indole	1/1000	Soluble in alcohol.	Alcohol conc. in the bath was 0.25% which had a slight inhibitory effect.
6-methyl indole	1/250	Soluble in alcohol.	Effect partly due to alcohol; conc. of alcohol in the bath was 0.25%
3-carb-ethoxy-5-methoxy-2-methyl indole.	$> \frac{1}{10}^{(X)}$	Soluble in warm alcohol.	25mg/l. maintained in the bath as lower conc. had no effect. Doses up to 5 $\mu$ g. of HT produced no contraction. Inhibition mostly due to alcohol, (conc. 1.2%)

TABLE IV Contd:

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ 1 hour)	Solubility	Remarks
1-3-indo- lyl-ethyl- 2 bromide	1/200	Soluble in warm alcohol.	
2-methyl- 3-acetyl indole	$> 2.5$	Very insoluble, saturated solution used.	In the conc. used ( $> 4\text{mg/l.}$ ) up to $20\mu\text{g}$ of HT never produced more than 50% of the maximum response. Acetylcholine like-wise inhibited.
2-Acetyl indole oxime	1/400	Soluble in warm alcohol.	
6-amino- 3-ethyl- 2-methyl indole	$> 1/5$ (X)	Soluble in warm acidic normal saline solution.	$1\text{mg/l.}$ potenti- ation of HT- response; $> 1\text{mg/l.}$ to $10\text{mg/l.}$ no potentiation or inhibition; $25\text{mg/l.}$ marked inhib- ition of both HT (up to $10\mu\text{g}$ ) and acetyl- choline.
7-amino- 3-ethyl- 2-methyl- indole	1/200	Soluble in alcohol.	Conc. of alcohol in bath 4.75%. Inhibitory effect mainly due to alcohol. $25\text{mg/l.}$ was maintained in the bath. Lower conc. had no effect.



TABLE IV Contd:

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) 1 hour	Solubility	Remarks
$\beta$ -indolyl aceto- nitrile	1/1000	Thick, oily liquid. Soluble in alcohol.	Effect partly due to alco- hol (1.7%)
1-methyl indole- 3-aceto- nitrile	$> 1/10^{(X)}$	Soluble in alcohol.	$\leftarrow$ 50mg/l. no effect. 50mg/l. maintained in the bath - no response to HT even when the dose was inc- reased to 10 $\mu$ g. Alcohol conc. 0.62% which partly contributed to the inhibition.
2-methyl- 3-indolyl- aceto- nitrile	1/1334	Soluble in alcohol.	
4-methyl- $\beta$ - indolyl- aceto- nitrile	1/1000	Soluble in alcohol.	
5-methyl indole- 3-aceto- nitrile	1/426	Soluble in alcohol.	
7-methyl indole- 3-aceto- nitrile	1/800	Soluble in alcohol.	
1-(3-indo- lyl)2- methyl- 2-nitro- propane	1/26	Soluble in alcohol.	
1-(3-indo- lyl)-2- nitro- butane	1/58.8	Thick brownish liquid. Soluble in alcohol.	

TABLE IV Contd:

Names of the compounds	Drug ratio  ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) 1 hour	Solubility	Remarks
3-ethyl-2-methyl-4-nitro indole	1/30.8	Soluble in alcohol.	
2:3-dimethyl-4-nitro indole	1/154	Soluble in alcohol.	5mg/l. maintained in bath. Alcohol conc. in bath 2.5% which has a slight inhibitory effect. The response was unusual. For the first $\frac{1}{2}$ hr there was marked inhibition, after which the effect seemed to decrease. E.g. after 40 mins. the dose ratio was 25, but after 1 hr it was 7.5
3-ethyl-2-methyl-5-nitro indole	(X) 1/5	Soluble in alcohol.	1-10mg/l. - no effect. 100mg/l. depressed the responses to HT (20 $\mu$ g); and acetylcholine markedly. Effect mainly due to alcohol (bath conc. 2.5%)

TABLE IV Contd:

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) - 1 hour	Solubility	Remarks
3-ethyl-2-methyl-6-nitro indole	$> 1/500^{(X)}$	Soluble in alcohol.	1-10mg/l. - no effect. 50mg/l. maintained in bath which contained alcohol conc. of 50%. Effect therefore, mainly due to alcohol.
2:3-dimethyl-6-nitro-indole	1/22	Soluble in alcohol.	Alcohol conc. in bath 1%, which has an inhibitory effect.
3-ethyl-2-methyl-7-nitro indole	1/2	Soluble in alcohol.	
3:5-dinitro-2-phenyl indole	1/40	Very insoluble; dissolved in alcohol + 2 drops of 0.5% sodium hydroxide.	Doses of 1-5mg/l. slight potentiation of HT response. Alcohol conc. in bath 0.95%. Effect thus in part due to alcohol.
7-amino-1:2:3:4-tetrahydro-carbazole	1/66	Soluble in warm acidic normal saline solution.	

TABLE IV Contd:

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) 1 hour	Solubility	Remarks
8-amino-1:2:3:4:tetrahydro carbazole	1/800	Soluble in normal saline	
1-nitro-carbazole	1/100	Soluble in warm alcohol.	
2-nitro-carbazole	1/26	Soluble in warm alcohol.	
3-nitro-carbazole	1/80	Soluble in warm alcohol.	
4-nitro-carbazole	1/12	Soluble in warm alcohol.	
5-nitro-tetrahydro-carbazole	1/10	Soluble in alcohol.	
6-nitro-tetrahydro-carbazole	1/88	Soluble in warm alcohol.	Alcohol conc. in bath 0.95% which inhibits the responses to both HT and acetylcholine.
7-nitro-tetrahydro-carbazole	1/40	Soluble in warm alcohol.	
8-nitro-tetrahydro-carbazole	$> 1/5^{(X)}$	Soluble in warm alcohol.	1-10mg/l.- no effect. 25mg/l. depressed sensitivity of preparation to a marked degree. HT (10 $\mu$ g) and acetylcholine - no response. Inhibition partly due to alcohol (bath conc. 2.3%)

TABLE IV Contd:

Names of the compounds	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of antagonist}}$ ) 1 hour	Solubility	Remarks
N-ethyl-N-1-naphthylmethyl-2-bromoethylamine (SY-28)	2	Easily soluble	
Dihydroergocristine-methanesulphonate	1.75	Soluble in hot normal saline solution.	
Mepyramine maleate	1/40	Easily soluble	
Diphenhydramine	1/80	Easily soluble	
Atropine sulphate	1/200	Easily soluble	

(X)

With these compounds, the drug ratio could not be accurately measured as the standard response of 50 per cent of the initial maximum effect could not be elicited in the highest concentration of 5-hydroxytryptamine employed in these experiments (5-20  $\mu\text{g}$  in 2 ml bath). These compounds were inactive in the concentrations which were used to find the drug ratios for other compounds.

The drug ratios at different concentrations were estimated for some of the specific and most active compounds. The results which are given in Table V appear to be more or less constant when low concentrations of these drugs were used. With higher concentrations of dibenamine, lysergic acid diethylamide, dihydroergotamine and 5-benzyloxy gramine, the drug ratio became very high. It appears that with these drugs there is a critical range of doses which gives a more or less constant drug ratio but when that range is exceeded, the highest concentration of the agonist used in the experiments either did not give any effect or failed to produce the standard response of 50 per cent of the maximum effect. Such a block may be called unsurmountable (surmountable for competitive, P.113. It has been described as non-competitive or absolute (Nickerson, 1949) and is similar to the type of antagonism where the log dose effect curve after the antagonist becomes flatter when compared with the original log dose effect curve (Schild, 1949). The difference in dose levels of these antagonists to produce a surmountable and an unsurmountable block was very narrow. Table V shows/



shows that a twofold increase in the concentration of dihydroergotamine used to give a surmountable effect caused an unsurmountable inhibition of the response to 5-hydroxytryptamine. However, dibenamine and 5-benzyloxy gramine also suppressed the response to acetylcholine in these high concentrations, while the two ergot alkaloids had no effect on its response. The effect of lysergic acid diethylamide and dihydroergotamine therefore remained specific even when the block became unsurmountable, while dibenamine and 5-benzyloxy gramine caused an unspecific effect with the unsurmountable block.

The unsurmountable type of effect was not possible to show with 5-methyl gramine as it produced stimulation of the uterus in higher concentrations (10 mg/l.). It rather showed a decrease in the drug ratio as is apparent from Table V when a surmountable effect was obtained with higher concentrations. The effect of acetylcholine was also inhibited with this high concentration of 5-methyl gramine. In a few cases where the experiments were repeated using the same concentration of the antagonist, the drug ratio after 1 hour gave about the/



TABLE V.

Drug ratios at different concentrations.

Names of the compounds	Concentrations $\mu\text{g/l.}$	Drug ratio ( $\frac{\text{conc. of HT}}{\text{conc. of an-}}$ ) tagonist 1 hour
Dibenamine hydrochloride	1/2	24-30
	10	35-50
	100 <sup>+</sup>	> 500
Lysergic acid diethylamide	1/2	16
	10	20
	100	> 150
Dihydroergotamine methanesulphonate	10	5
	50	4-5
	100	> 100
5-benzyloxy gramine	100	1/3.2
	1000	1/2.5
	5000 <sup>+</sup>	> 2
5-methyl gramine	200	1/7.4
	500	1/8.4-1/7.2
	1000	1/10
	3000 <sup>+</sup>	1/17 - 1/15

<sup>+</sup>With these concentrations, the response to acetylcholine was also depressed and the effect therefore was unspecific.

the same figure. Most of the compounds studied in the present series showed an unspecific effect. A large number of these compounds were very insoluble and the effect of alcohol (solvent) interfered with some of the results. However, 2-acetyl indole oxime, aceto nitrites, amino indoles and carbazoles and nitro carbazoles inhibited the response to 5-hydroxytryptamine to a much greater extent than the response to acetylcholine.

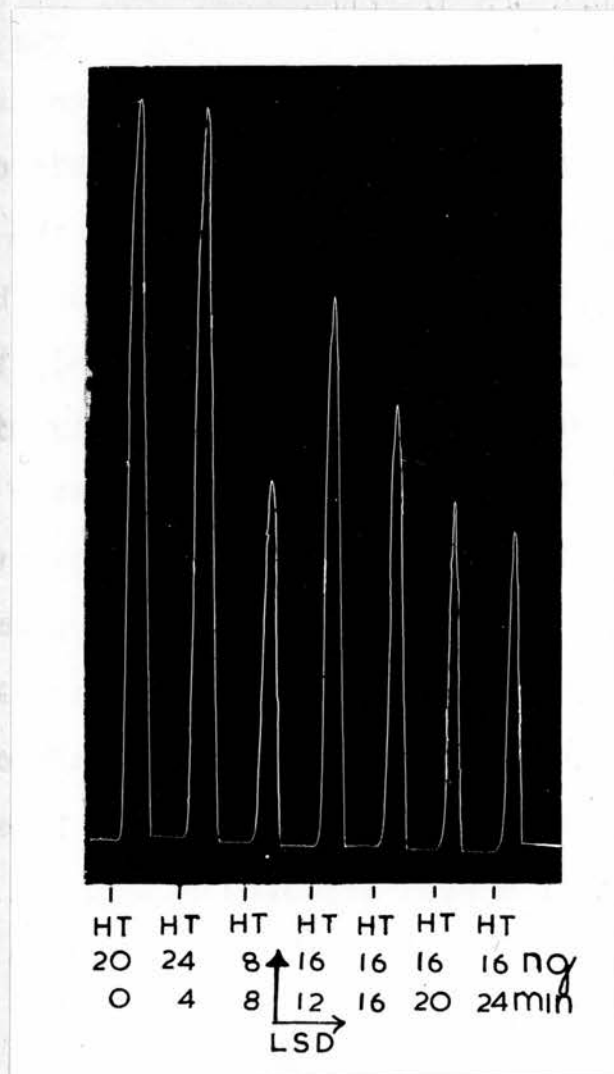
#### The $pA_x$ .

Some of the more potent drugs detected by the method described above were then studied in more detail and estimates made of the  $pA_2$ .

When curves have been obtained like those in Figs. 35 and 36 showing the relation between the dose ratio and time, it is possible to read directly from the curve the time corresponding to any given dose ratio ( $x$ ), and thus to calculate the  $pA_x$  corresponding to this time.

The  $pA_2$  was estimated by a method rather different from that used by Schild (1947). A response equal to 50 per cent of the maximum effect was first obtained/

Figure 42



Rat's uterus - 2ml. bath

Measurement of  $pA_2$

HT - 5-hydroxytryptamine

LSD - lysergic acid diethylamide  
( $1\mu\text{g}/\text{l.}$ ) added to de Jalon's  
solution at 9 min.

obtained to a dose of 5-hydroxytryptamine, and then double this dose of 5-hydroxytryptamine was applied in the presence of a suitable concentration of the antagonist. This double dose of 5-hydroxytryptamine was repeated at intervals and the  $pA_2$  value was taken at a time when the response to the double dose of 5-hydroxytryptamine in the presence of the antagonist was equal to the original effect produced by a single dose. When the responses were not equal, the approximate time, when the effect was slightly higher before and slightly lower after, was taken for the  $pA_2$  value. Fig.42 shows how these estimates were made. A dose of 20 ng of 5-hydroxytryptamine produced a maximum effect and a response equal to 50 per cent of the maximum effect was obtained with a dose of 8 ng of 5-hydroxytryptamine. Lysergic acid diethylamide in a concentration of 1  $\mu\text{g/l}$ . was then added to the bath at 9 min. and maintained thereafter. A dose of 16 ng of 5-hydroxytryptamine was then applied and repeated at intervals. The response to the double dose of 5-hydroxytryptamine after 7 min. of the application of lysergic acid diethylamide was higher and after 11 min. it was lower than the original/

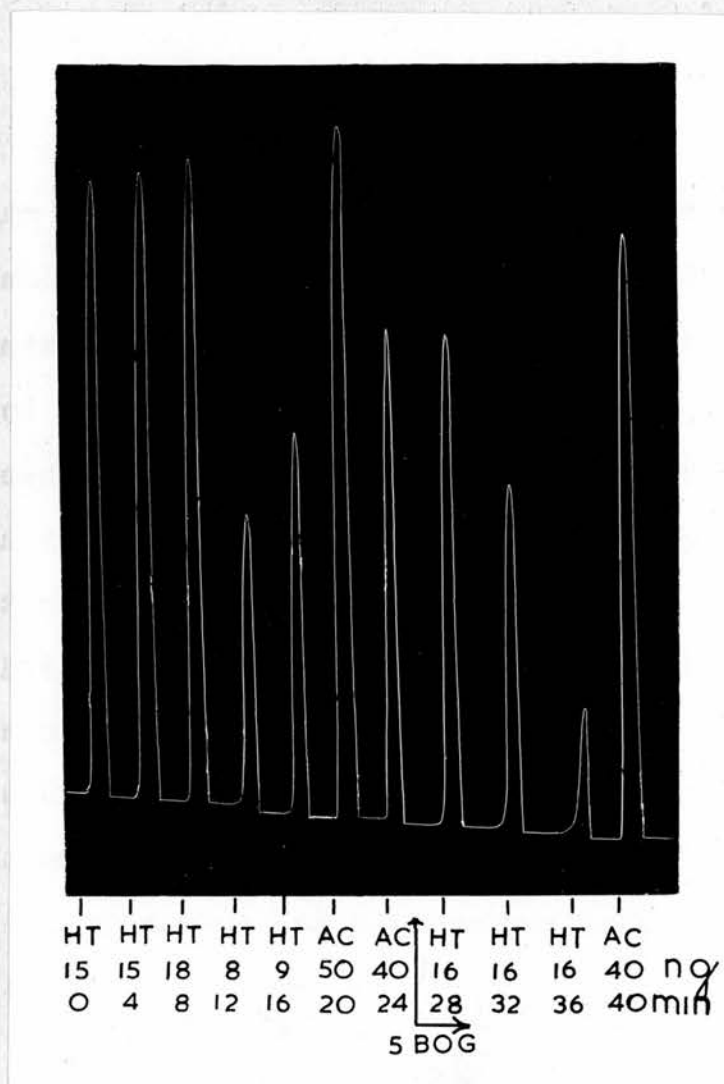
original effect of a single dose. The  $pA_2$  for lysergic acid diethylamide was therefore taken after 9 minutes to be  $(10^{-9})$  8.5 (mol. wt. of LSD 323).

A similar experiment with 5-benzyloxy gramine is illustrated in Fig. 43. In this experiment, a dose of 18 ng of 5-hydroxytryptamine produced a maximum effect and a dose of 8 ng of 5-hydroxytryptamine gave a response which was 50 per cent of the maximum effect. The control drug, acetylcholine, in a dose of 40 ng caused a submaximal response. 5-benzyloxy gramine in a concentration of 100  $\mu\text{g/l}$ . was maintained in the bath continuously from 25 minutes and doses of 16 ng of 5-hydroxytryptamine applied at intervals. The response to this double dose of 5-hydroxytryptamine was higher after 7 minutes and smaller after 11 minutes of the exposure to the antagonist when compared to the original effect of a single dose. The  $pA_2$  for 5-benzyloxy gramine was taken after 9 minutes as  $(10^{-7})$  6.44 (mol. wt. of 5-benzyloxy gramine 280).

Such results cannot of course, be used directly to compare one drug with another because the time corresponding to each estimate varies uncontrollably.

As/

Figure 43



Rat's uterus - 2 ml. bath

Measurement of  $pA_2$

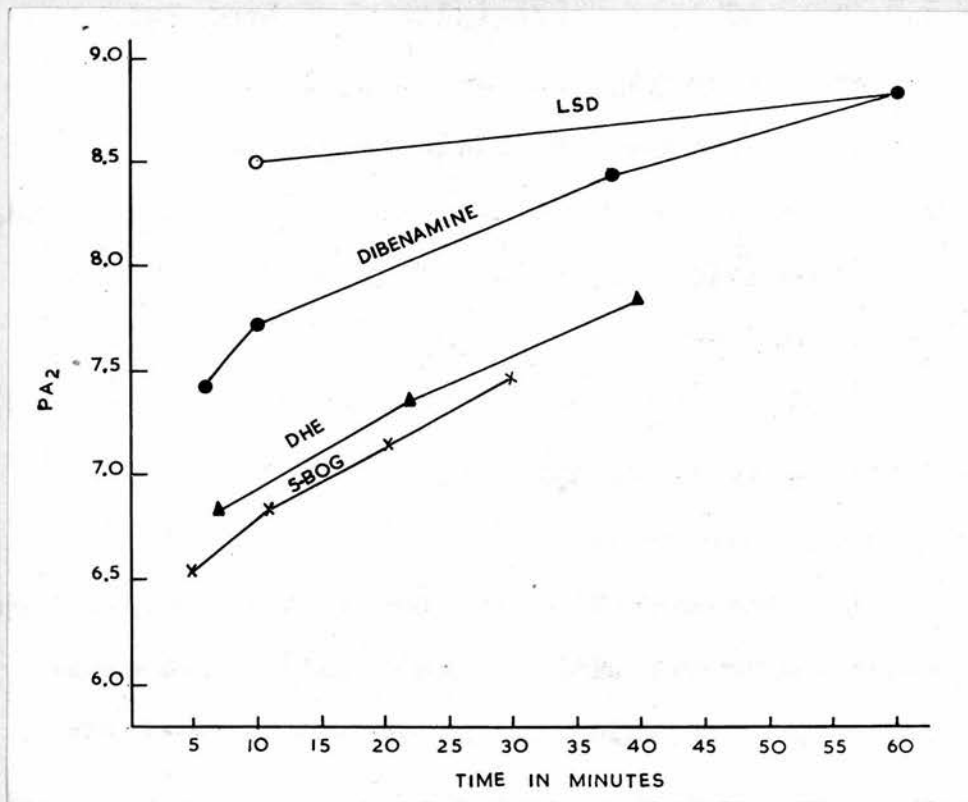
HT - 5-hydroxytryptamine

AC - acetylcholine

5-BOG - 5-benzyloxy gramine (100  $\mu$ g/l.)  
added to de Jalon's solution at  
25 min.



**Figure 44**



The relation of pA<sub>2</sub> to time.

LSD - lysergic acid diethylamide	○ ————— ●
Dibenamine	● ————— ●
DHE - dihydroergotamine	▲ ————— ▲
5-BOG - 5-benzyloxygramine	x ————— x



As the effects of dibenamine, lysergic acid diethylamide, dihydroergotamine and 5-benzyloxy gramine continued to increase for an hour or so, the estimates of  $pA_2$  were made at different time intervals up to 1 hour. When, however, a series of different concentrations of these antagonists had been used, the relation between  $pA_2$  and time was plotted as shown in Fig. 44. The  $pA_2$  for these drugs continued to increase for at least 30 minutes. From such curves, the  $pA_2$  corresponding to an arbitrarily fixed time can be determined.

The  $pA_2$  values for dihydroergokryptin, 5-methyl gramine, mepyramine, diphenhydramine and atropine were also determined.

As  $pA$  measurements are more variable after long periods of contact with the antagonist than after short periods (Schild, 1947), 10 minutes contact with the antagonist was considered to give a reliable result. Table VI gives the  $pA_2$  values for various compounds after 10 minutes contact.

TABLE

TABLE VI.Results of  $pA_2$  determinations.

Names of the Compounds	$pA_2$ after 10 min. contact.
Lysergic acid diethylamide (LSD)	8.5
Dibenamine	7.72
Dihydroergotamine	6.9
Dihydroergokryptin	6.82
5-benzyloxy gramine	6.79
5-methyl gramine	6.57
Mepyramine	6.3 (6 min.)
Diphenhydramine	5.86
Atropine	5.84 (4 min.)

$pA_2$  for the last three drugs reached a steady value in less than 10 minutes. This is in accord with the behaviour of non-specific and less active antagonists (Schild, 1947). The  $pA_2$  gave fairly constant results when the experiments were repeated. The  $pA_2$  estimate of 8.5 for lysergic acid diethylamide/

*agrees well*  
diethylamide corresponds favourably with the figure of 8.7 as reported by Gaddum (1953b) for the same compound.

Stimulation followed by specific depression of the tryptamine receptors.

The 12 indole compounds shown in Table VII caused stimulation of the uterus but this was followed by a specific depression of the tryptamine receptors. These drugs were kept in the bath for 10 minutes in the concentrations given in the table and then washed out. When the muscle had relaxed and the spontaneous activity ceased, it gave a diminished response to 5-hydroxytryptamine and a normal response to acetylcholine. The response to 5-hydroxytryptamine gradually recovered. These compounds thus behaved like 5-hydroxytryptamine itself which also causes stimulation followed by depression of the tryptamine receptors (Gaddum, 1953a). Such types of experiments were difficult to reproduce with gramine methosulphate, 2-methyl gramine and bufotenin. They caused marked contraction and spontaneous activity which persisted for a long time when these drugs were removed from the bath. Most of/

of these drugs in low concentrations, when they did not cause any contraction potentiated the response to 5-hydroxytryptamine. Such potentiation of the response to 5-hydroxytryptamine was especially observed with gramine methosulphate, 2-methyl gramine and 6-methyl tryptamine in concentration of 10 mg/l.

The three gramine derivatives usually caused a good contraction in a concentration of 5-10 mg/l. They initiated much spontaneous activity of the uterus which interfered with the experiments. Occasionally, these compounds did not give any contraction but when kept for more than 10 minutes in the bath started the muscular activity. When their stimulant effects were compared with that of 5-hydroxytryptamine on the rat's uterus, they were about 500-1000 times less active than 5-hydroxytryptamine.

Bufotenin in a dose of 100-300  $\mu$ g/l. gave a satisfactory response and was 15-40 times less active than 5-hydroxytryptamine on the rat's uterus. Erspamer (1952c) found bufotenin 1/10 as active as enteramine on the rat's uterus.

6-hydroxytryptamine was very feeble in its action. A dose of 100 mg/l. elicited a response on the/

TABLE VII.Specific, but stimulant indole compounds

Names of the compounds	Conc. in the bath for 10 min. to inhibited HT. mg/l.
Gramine methosulphate	50-100
2-methyl gramine	50
5-hydroxy gramine	5-10
Bufotenin (N-dimethyl-5 hydroxy- tryptamine) creatinine sulphate	10
6-hydroxytryptamine	100
1-methyl tryptamine	10
4-methyl tryptamine	10
5-methyl tryptamine	10
6-methyl tryptamine	10
7-methyl tryptamine	1-10
$\alpha$ -methyl tryptamine	10
N-isopropyl tryptamine	10

the uterus and was about 10,000 times less active than 5-hydroxytryptamine. The tryptamines usually gave a satisfactory response in concentrations of 5-15 mg/l.

5-hydroxytryptamine/

5-hydroxytryptamine was 500-1500 times more active than the various tryptamine derivatives. Erspamer (1952c) reported 1-methyl tryptamine to be about 100 times less active than 5-hydroxytryptamine when tested on the rat's uterus. Tryptamine itself was more active than its methyl and isopropyl derivatives. Among the methyl tryptamines themselves, the few observations which were made indicated that 5-methyl tryptamine was slightly more active than the other methyl derivatives. There was not much difference between the activities of 1, 4, 6 and 7-methyl and  $\alpha$ -methyl tryptamines. N-isopropyl tryptamine, however, seemed to be comparatively less active than the methyl tryptamines.

The initial stimulant effect of these compounds was also found to be specific, since it could be specifically antagonised by small doses of other indole compounds which had been found to be specific antagonists of 5-hydroxytryptamine.

Since the three derivatives of gramines occasionally, did not cause contractions, the inhibition of their stimulating effect by other known antagonists of 5-hydroxytryptamine was not studied.

The response to bufotenin was suppressed by 5-benzyloxy-/

5-benzyloxy gramine (500  $\mu\text{g}/\text{l.}$ ) and dihydroergotamine (100  $\mu\text{g}/\text{l.}$ ). The effects of 6-hydroxytryptamine, 1, 5, 6 and 7-methyl tryptamines were blocked by 5-methyl gramine in a concentration of 1  $\text{mg}/\text{l.}$

The response to 4-methyl tryptamine,  $\alpha$ -methyl tryptamine and N-isopropyl tryptamine were abolished by 4-methyl gramine (1  $\text{mg}/\text{l.}$ ).

Stimulation followed by unspecific depression of the receptors.

The 10 compounds shown in Table VIII caused stimulation of the uterus in the concentration used but this was followed by unspecific depression of the uterus as the response to acetylcholine was also inhibited.

These drugs were kept in the bath for 10 minutes usually in concentrations given in the table and then washed out. When the muscular contractions had ceased, the subsequent direct effects of both 5-hydroxytryptamine and acetylcholine were diminished. Concentrations lower than those shown in the table neither caused any contractions nor had any effect on the responses to 5-hydroxytryptamine and acetylcholine.

2 and 3 aminocarbazoles and tetrahydroharman did/



TABLE VIIIUnspecific stimulant indole compounds.

Names of the compounds	Conc. which caused stimulation. mg/l.
4-amino-3-ethyl-2-methyl indole	50
4-amino-2:3-dimethyl indole	50
7-amino-2:3-dimethyl indole	25
2:3-dimethyl-7-nitro indole	1
3-carbethoxy-6-nitro indole	1
1-aminocarbazole	10
2-aminocarbazole	10
3-aminocarbazole	10
5-amino-1:2:3:4-tetrahydro-carbazole	25
Tetrahydroharman	10

did not show much inhibition of the effects of 5-hydroxytryptamine in concentrations of 10 mg/l. but when higher concentrations (25 mg/l.) were used, no stimulant effect was observed and the responses to both 5-hydroxytryptamine and acetylcholine were markedly inhibited. Even increasing the dose of 5-hydroxytryptamine to 400-2000-fold did not produce any effect.

7-amino 2:3-dimethyl indole produced slight inhibition of the effects of 5-hydroxytryptamine and acetylcholine in the concentrations used (25 mg/l.). The two 4-amino indoles showed a delayed stimulant effect. The muscle started contracting gradually after the drugs had been in the bath for about 8 minutes. It went into a marked spasm and took 45 minutes to relax after the drugs had been removed from the bath.

#### Reversibility.

The recovery of the responses to 5-hydroxytryptamine after the antagonists were removed from the bath was observed for 1-2 hours before it was deemed irreversible or only partially reversible. The recovery of the response to 5-hydroxytryptamine was/

was dependent on the concentration of the antagonist used and on the length of time the antagonist was in contact with the muscle. In general, these effects were studied with the various antagonists in the concentrations used for finding the ratios, (Table III and IV). In most cases, the time of contact with the <sup>muscle</sup> uterine/ was 1 hour.

Reversibility of specific antagonists.

The effects of dibenamine, lysergic acid diethylamide, dihydroergotamine and the various other ergot alkaloids were partially reversible when used in low concentrations (1-10  $\mu\text{g}/\text{l.}$ ) but higher concentrations ( $> 10 \mu\text{g}/\text{l.}$ ) produced an irreversible block. Dibenamine, however, seemed to be the most potent as the response to 5-hydroxytryptamine showed very slight recovery even when low concentrations of dibenamine were employed.

The antagonistic actions to the response to 5-hydroxytryptamine, of the benzyloxy gramines, were reversible in concentrations of 10  $\mu\text{g}/\text{l.}$ , partially reversible in concentrations of 1  $\text{mg}/\text{l.}$  and irreversible in higher concentrations (5  $\text{mg}/\text{l.}$ ).

The/

The methyl gramines showed a reversible effect in concentrations of 10-100  $\mu\text{g}/\text{l.}$ , but with concentrations of 1  $\text{mg}/\text{l.}$  there was partial recovery of the response to 5-hydroxytryptamine.

The sensitivity of the uterine muscle to 5-hydroxytryptamine slowly recovered after the various tryptamine derivatives were removed from the bath.

In the case of 5-amino-3-ethyl-2-methyl indole, the recovery was almost immediate. On the other hand, the response to 5-hydroxytryptamine after 6-aminotetrahydrocarbazole was removed, showed a gradual recovery to the original level.

Reversibility of unspecific antagonists.

In most of these cases, the effects of the control drug, acetylcholine, recovered more rapidly than the responses to 5-hydroxytryptamine. After treatment of the muscle with the various methyl derivatives of indole, the response to 5-hydroxytryptamine showed a slow recovery to normal in every case.

The antagonistic effect of 3-carbethoxy-5-methoxy-2-methyl indole proved to be readily reversible in contrast to that of 2-methyl-3-acetyl indole from which no recovery was obtained.

In/

In the series of indole derivatives containing an amino group attached to the ring, only 7-amino-3-ethyl-2-methyl indole produced an inhibition of 5-hydroxytryptamine response from which complete, albeit slow, recovery was observed. The other amino derivatives exerted antagonistic effects which were irreversible or only partially reversible.

The inhibitory actions of the acetonitriles proved to be fleeting since the muscle rapidly regained its sensitivity to 5-hydroxytryptamine after removal of the antagonists from the bath. It is interesting to note that with  $\beta$ -indolyl acetonitriles the sensitivity of the muscle to 5-hydroxytryptamine returned to the original level more rapidly than did the sensitivity to acetylcholine.

The nitro indole series of compounds were found to produce irreversible or only partially reversible block of the 5-hydroxytryptamine response. In all cases, the acetylcholine contractions showed a gradual recovery.

With all the carbazole derivatives tested, an inhibition of the 5-hydroxytryptamine response was obtained. On removal of the antagonist, the response level returned to normal in every case but the/

the rate of the recovery varied. Gradual recoveries were obtained with the aminotetrahydrocarbazoles and the nitrocarbazoles while with the tetrahydro-derivatives of the latter the recoveries were more rapid.

N-ethyl-N-1-naphthyl-methyl-2-bromo ethylamine (SY-28) and dihydroergocristine behaved in a manner similar to dibenamine and to other lysergic acid derivatives in so far as the recoveries of the 5-hydroxytryptamine responses were concerned.

The response to 5-hydroxytryptamine gradually recovered to its original level when mepyramine, diphenhydramine or atropine were washed out of the bath.

After treatment with the compounds which caused stimulation followed by specific depression of the tryptamine receptors (Table VII ) the uterine muscle showed gradual recovery of its sensitivity to 5-hydroxytryptamine.

The inhibition of the response to 5-hydroxytryptamine was partially reversible or irreversible with those compounds which caused stimulation followed by unspecific depression. After the aminocarbazoles, partial recovery was obtained but with the amino- and nitro-indoles and tetrahydroharman the effects were irreversible.

The/

The above mentioned recovery results showed that most of the compounds which reached equilibrium within a short time were rather inactive and their effects were easily reversible. Some of the unspecific alcohol soluble compounds in the present series showed marked inhibitory effects, but when they were washed out, there was rapid recovery of the responses to 5-hydroxytryptamine and acetylcholine. Such effects were probably due to alcohol.

Chemical structure and pharmacological action.

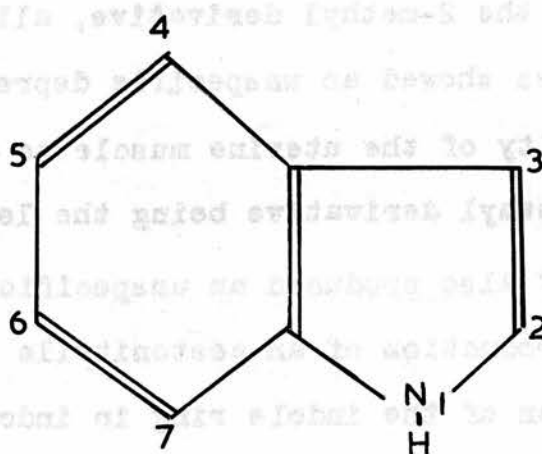
The indole compounds tested in this study were derivatives of indole itself, or of gramine or of tryptamine. In addition, a number of carbazole compounds and lysergic acid derivatives were investigated. These too, may be regarded as indole derivatives since the indole nucleus comprises part of the ring structure of these compounds.

The introduction of various substituent groups into the parent molecule was found to effect the pharmacological action to a degree which depended not only on the nature of the group introduced but also on its position in the molecule. To simplify discussion of these points each class of compound will/



Figure 45

INDOLE



will be considered separately. At the outset, it should be pointed out that many of the series were incomplete and the conclusion drawn should therefore, be regarded only as tentative.

### Indoles (Fig. 45).

The Mono Methyl indoles. With the exception of the 2-methyl derivative, all the mono methyl indoles showed an unspecific depression of the sensitivity of the uterine muscle to agonists, with the 5-methyl derivative being the least active. Indole itself also produced an unspecific effect.

The introduction of an acetonitrile group into the 3-position of the indole ring in indole and the methyl indoles led to a diminution in activity which was still unspecific. The most active of the acetonitriles was 5-methyl-3-indolylacetonitrile; the least active being the 1-methyl derivative. The last substance showed no activity in the ordinary concentration (10 mg/l.) in which the other members of the series showed some activity but when the concentration was raised to 50 mg/l. had a marked inhibitory effect on the 5-hydroxytryptamine responses. It is interesting to note that the acetonitriles were found/

found to produce their maximum inhibitory effect immediately which, in turn, was immediately abolished on removing the antagonist from the organ bath.

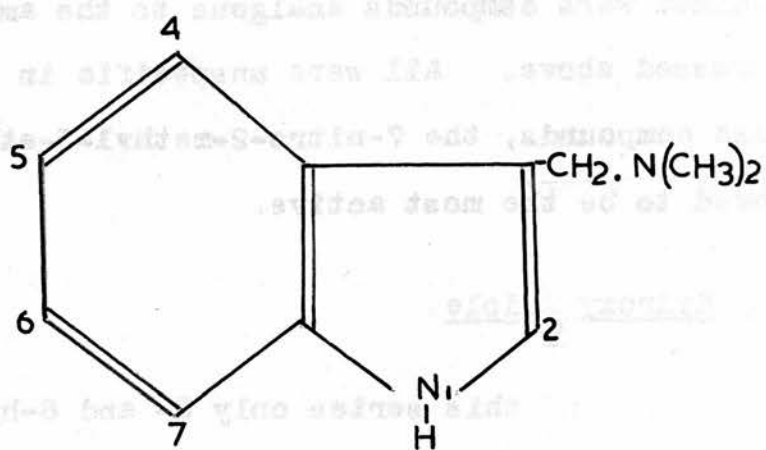
#### Amino indoles.

No monoamino indole compounds were available for study. The amino indoles examined were homologues of the compounds shown by Woolley and Shaw (1953c) to have an antagonistic effect on the action of 5-hydroxytryptamine on carotid artery strips. Of the amino derivatives of 2-methyl-3-ethyl indole, only the 5-amino compound showed a specific antagonistic action. The other compounds exhibited an unspecific effect, with the 4-amino derivative showing, in addition, a stimulant action on the uterus.

In the series of amino derivatives of 2:3-dimethyl indole the 6-amino compound showed a specific antagonistic action which was more intense than that of the 5-amino-2-methyl-3-ethyl compound. Unfortunately, the 5-amino derivative of this series was not available for examination. The other members of the series, 4-amino and 7-amino caused a contraction of/

Figure 46

GRAMINE



of the uterus and an unspecific depression of the muscle responses to the agonists.

#### Nitro indoles.

No mononitro indoles were available for study. Structurally, most of the nitro indoles examined were compounds analgous to the amino indoles discussed above. All were unspecific in action. Of these compounds, the 7-nitro-2-methyl-3-ethyl indole proved to be the most active.

#### Hydroxy indole.

Of this series only 5- and 6-hydroxy-indole were tested. Neither of these showed any significant action.

#### Gramine (Fig.46) derivatives.

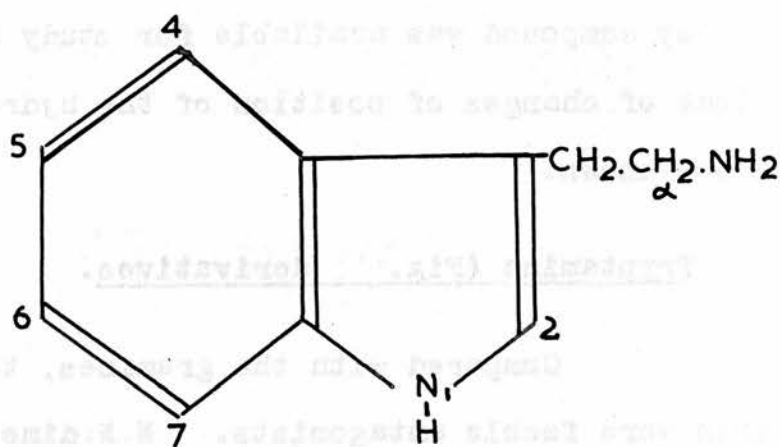
These were all good antagonists, the 5-benzyloxy gramine showing the greatest activity. The benzyloxy derivatives were found to be more active than the corresponding methyl derivatives. While the maximum inhibitory action of the methyl gramines was reached within 30 minutes contact with the preparation/

preparation, that of the benzyloxy gramines developed more slowly, taking 1 hour at least. The position of these groups in the ring was important; the substitution in position 5 had the maximum effect while 6-methyl gramine was more active than 4-methyl gramine. Substitution of the hydrogen atom on the indole nitrogen by a methyl group decreased the activity. Gramine itself first had a weak inhibitory effect on the 5-hydroxytryptamine responses but this was soon masked by spontaneous contractions of the muscle some 10 minutes after the gramine had been introduced into the bath. The 2-methyl gramine and gramine methosulphate produced first, stimulation of the muscle and after removal from the bath the response to 5-hydroxytryptamine was inhibited. In low concentrations where these drugs did not cause stimulation, the response to 5-hydroxytryptamine was potentiated in the presence of these substances.

The substitution of a methyl group in the benzene ring portion of the indole structure of gramine produced compounds which were antagonistic to the action of/

Figure 47.

## TRYPTAMINE





of 5-hydroxytryptamine without causing preliminary stimulation of the muscle by their own action.

5-hydroxy gramine unlike 5-methyl gramine or 5-benzyloxy gramine caused contraction of the uterus in the doses required to produce an inhibitory effect of the 5-hydroxytryptamine response. No other hydroxy compound was available for study and so the effect of changes of position of the hydroxyl group is not known.

Tryptamine (Fig.47) derivatives.

Compared with the gramines, the tryptamines were feeble antagonists. N:N:dimethyl tryptamine, the homologue of gramine, was the most active in this series. This substance had more activity than that containing two methyl groups or an ethyl group on the  $\alpha$ -carbon atom. In the concentrations used, none of these compounds per se, produced stimulation of the muscle.

In the series of compounds resulting from the introduction of a methyl group into the indole ring of tryptamine, the position of the methyl group was again a determining factor in the pharmacological action./

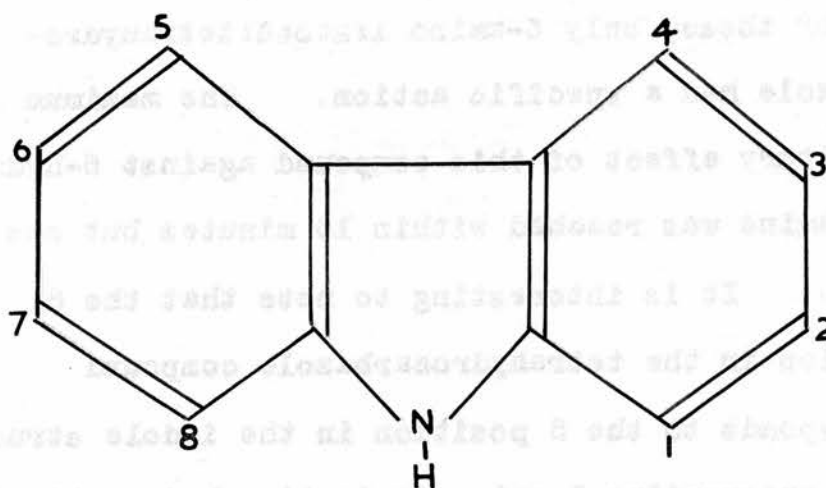
action. The compound with the methyl group in position 2 was inhibitory to the 5-hydroxytryptamine response and without stimulant effects on the uterus. A methyl group in any other position on the indole nucleus of tryptamine resulted in compounds which caused contraction of the muscle. The substitution of a methyl group in position 5 produced a compound which was more potent for its stimulant action on the uterus than the other methyl derivatives of tryptamine.

It thus appeared that of the compounds resulting from the introduction of a methyl group into the various positions of the indole ring of gramine, only the 2-methyl derivative was a uterine stimulant, while, in the tryptamine series, uterine stimulation was a property only of those derivatives in which the methyl group occupied a position other than the 2 position in the indole ring.

In contrast to the marked stimulant effect of 5-hydroxytryptamine on the uterus, 6-hydroxytryptamine was to all intents and purposes, inactive. Like 5-hydroxytryptamine itself, high doses of the 6-hydroxy derivative desensitized the muscle to the action/

Figure 48

CARBAZOLE



action of 5-hydroxytryptamine. Compounds with the hydroxyl group in other positions were not examined.

Carbazoles (Fig.48).

In the carbazole series, only amino and nitro derivatives of carbazole and 1:2:3:4:tetrahydrocarbazole have been studied.

Of these, only 6-amino 1:2:3:4:tetrahydrocarbazole had a specific action. The maximum inhibitory effect of this compound against 5-hydroxytryptamine was reached within 10 minutes but was feeble. It is interesting to note that the 6 position in the tetrahydrocarbazole compound corresponds to the 5 position in the indole structure. The corresponding 5-amino derivative had a stimulant action on the uterus which was lacking with the 6, 7, and 8-amino compounds. The 5,7 and 8-amino derivatives showed an unspecific decrease of the response to 5-hydroxytryptamine.

The corresponding nitro-1:2:3:4:tetrahydrocarbazoles showed no stimulant action but possessed an unspecific antagonistic action to the response of/

of the uterus to 5-hydroxytryptamine. The 5-nitro compound proved to be the most potent.

The analagous monoaminocarbazoles all possessed a stimulant action not shown by the corresponding nitro derivatives. Both the amino and nitrocarbazoles were unspecific inhibitors of 5-hydroxytryptamine.

Some of the apparently anomalous findings observed, particularly with the unspecific compounds might be due to the fact that most of these compounds were very insoluble and the effect of the solvent used (alcohol) might have interfered with the result.

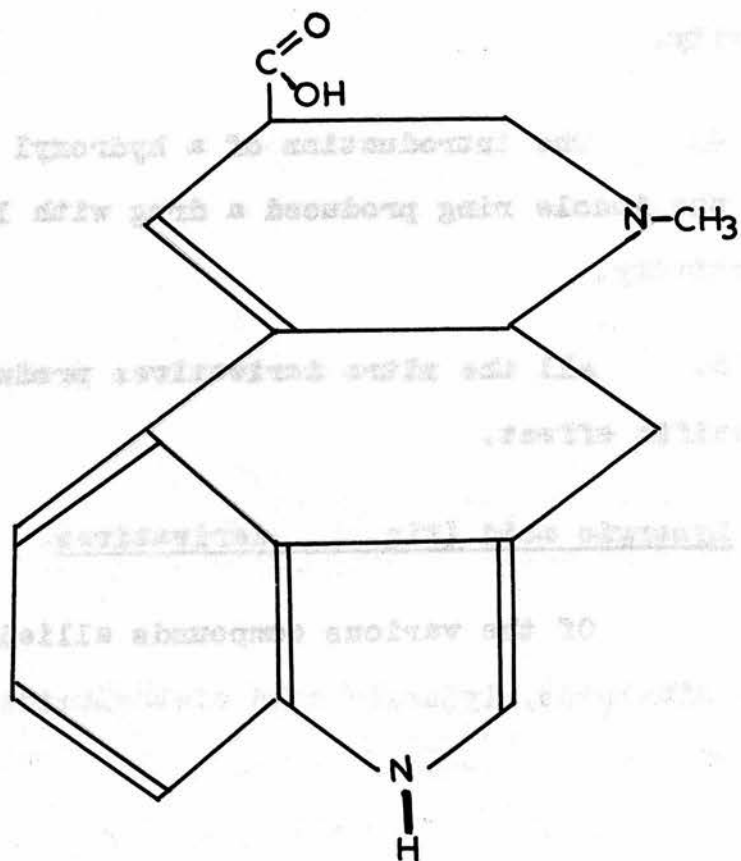
However, the above study of structure-activity relationship of the various indole and carbazole compounds revealed the following characteristic features for their anti-5-hydroxytryptamine actions.

1. The presence of the side chains of gramine and tryptamine compounds were important both for specificity and potency of inhibitory action against 5-hydroxytryptamine.

2. In most of the cases, the substitution of the 5 position in the indole ring (or the corresponding 6 position in tetrahydrocarbazole) by a methyl or/

Figure 49

LYSERGIC ACID



or an amino group increased the specificity and the activity of the compounds. Next in order was substitution in the 6 position.

3. Substitution of the hydrogen atom on the indole nitrogen by a methyl group decreased the activity.

4. The introduction of a hydroxyl group into the indole ring produced a drug with little or no activity.

5. All the nitro derivatives produced an unspecific effect.

Lysergic acid (Fig. 49) derivatives.

Of the various compounds allied to the ergot alkaloids, lysergic acid diethylamide was the most active and ergometrine the least. Table IX shows the composition of the various derivatives of lysergic acid tested.

Again, a comparison of the lysergic acid derivatives showed that the composition of the side chain attached to the carboxyl group mainly determined the anti-5-hydroxytryptamine activity. The/



TABLE IX.Composition of the lysergic acid derivatives.

Lysergic acid diethylamide	Lysergic acid	diethylamide
Dihydroergotamine	Dihydro Lyser- gic acid NH <sub>3</sub> Pyruvic acid d-proline	+ 1-phenylala- nine
Dihydroergokryptine	Dihydro Lyser- gic acid NH <sub>3</sub> Dimethyl-pyru- vic acid d-proline	+ 1-leucine
Dihydroergocornine	Dihydro Lyser- gic acid NH <sub>3</sub> Dimethyl-pyru- vic acid d-proline	+ 1-valine
Dihydroergocristine	Dihydro Lyser- gic acid Dimethyl-pyru- vic acid d-proline	+ 1-phenylala- nine
Ergometrine	Lysergic acid	Propan-1-ol-2- amide

The diethylamide group, as in lysergic acid diethylamide had the maximum effect while the propan-1-ol-2-amide group as in ergometrine had little effect.

The inhibitory actions of the other lysergic acid derivatives seemed to depend mainly on the polypeptide moiety which is present in these compounds and absent in ergometrine.

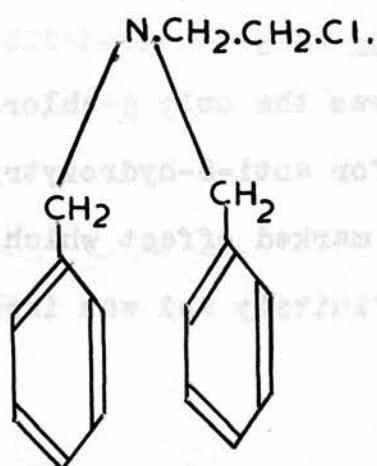
The difference in the potencies of dihydroergotamine from the other dihydro derivatives of lysergic acid might be due to the variations in amino acid constitution. The fact that the potencies of dihydroergotamine and dihydroergocristine were greater than those of dihydroergocornine and dihydroergokryptine suggests that the presence of a phenylalanine group in the molecule enhanced the anti-5-hydroxytryptamine activity.

The reason for the unspecific action of dihydroergocristine and the narrow range of specificity with dihydroergocornine (specific in concentrations  $< 50 \mu\text{g/l.}$ ) is not readily apparent. Perhaps the presence of dimethyl pyruvic acid in these compounds has some bearing on this.

Comparison/

Figure 50

DIBENAMINE



Comparison of the potencies of ergotamine and dihydroergotamine indicated that hydrogenation increased the 5-hydroxytryptamine blocking activity. This is also true for their anti-adrenaline action (Nickerson, 1949).

Dibenamine (Fig. 50) (N,N-dibenzyl- $\beta$ -chloroethyl-amine) was the only  $\beta$ -chloroethylamine compound tested for anti-5-hydroxytryptamine activity. It had a very marked effect which continued to increase indefinitely and was irreversible.

#### Discussion.

The estimation of the potencies of the various antagonists after an interval of 1 hour might be criticised because of the possible change of sensitivity of the preparation of 5-hydroxytryptamine after such a long interval. The dose-effect curves of 5-hydroxytryptamine were repeated at different intervals up to 1 hour on an ordinary preparation/

preparation and no significant difference was found between these curves.

Most of these measurements were taken when the equilibrium states between the agonist and the antagonist had been established but in a few instances, especially with some of the most active compounds it was not possible to be sure that the effect was complete after 1 hour. Failure to establish equilibrium conditions may make it difficult to distinguish between the effects of the antagonist and that of deterioration of the preparation, but in the present study, the control drug, acetylcholine, was often applied at intervals to test the sensitivity of the preparation. The sensitivity to acetylcholine did not diminish after 1 hour in the presence of these antagonists in the concentrations used.

The potency estimates of the most active compounds when repeated had been found to give constant results with the  $PA_{50}$  method. According to these estimates, lysergic acid diethylamide, dibenamine, dihydroergotamine, dihydroergokryptin and 5-benzyloxygramine were the most active antagonists of 5-hydroxytryptamine, on the rat's uterus (in descending order of/

of potencies). Dihydroergocornine had more activity than dihydroergokryptine but was only specific in concentrations lower than 50  $\mu\text{g/l}$ .

Among these compounds, estimates of dose ratio, drug ratio and  $\text{pA}_2$  agree, except that dibenamine gave a higher drug ratio but lower  $\text{pA}_2$  value than lysergic acid diethylamide. This difference in results might be due to the rate of development of the blockade at various concentrations of dibenamine. In low concentrations (1-5  $\mu\text{g/l}$ .) used for finding the  $\text{pA}_2$  values, dibenamine was less active than or as active, as lysergic acid diethylamide, while at the higher concentrations (10  $\mu\text{g/l}$ .) used for the drug ratio determination it was more active than lysergic acid diethylamide. Again, the effect of dibenamine seemed to develop very gradually in low concentrations as compared with that of lysergic acid diethylamide. Though the  $\text{pA}_2$  value of dibenamine was low at 10 minutes, it rose to be equal to that of lysergic acid diethylamide after 1 hour (Fig.44).

The study of time-action, concentration-action and recovery effects showed that there were two types of compounds. The most active compounds, such as/

as dibenamine, lysergic acid derivatives and the benzyloxy gramines, had an inhibitory action the intensity of which increased with time of contact with the muscle and which was irreversible or only partly reversible. In low concentrations they showed a surmountable block but at slightly higher concentrations of these antagonists, the inhibition of the response to 5-hydroxytryptamine became unsurmountable.

The other type included the methyl gramines, tryptamines, 5-amino-3-ethyl-2-methyl indole and 6-aminotetrahydrocarbazole. Their actions were complete within 30 minutes, were less intense and were easily reversible.

The above characteristics of the two types of compounds have been more or less, the rule in the present study. The blockade with the most active compounds developed gradually and the rate and degree of blockade depended upon the concentrations of the drug used.

It appears that these drugs have a great affinity for the tryptamine receptors and once they reach their site of action in a suitable concentration, it is difficult to dislodge them. However, an/



an alternative explanation for their effects may be that in low concentrations, like the less active compounds, they merely occupy the tryptamine receptors but in high concentrations they may poison this structure or some other essential mechanism which is concerned in the action of 5-hydroxy-tryptamine. This damaged system takes, perhaps, a long time for re-synthesis which might explain the observed irreversibility of their effects. Whatever may be the explanation of the mechanism of their actions, the various lysergic acid derivatives tested, with the exception of dihydroergocristine, seems to act specifically on the tryptamine receptors even in the state of unsurmountable block in the concentration used, while dibenamine and 5-benzyloxy gramine extend their toxic effects to other parts of the muscle so that the block becomes unspecific.

Lysergic acid diethylamide was thus the most specific and the most potent antagonist for 5-hydroxy-tryptamine on the rat's uterus. This substance has also other interesting pharmacological actions. It exerts the powerful mental effects in the human being (Stoll, 1947). In certain subjects, very small doses/

doses (0.02 mg) of lysergic acid diethylamide given orally, produce alternate phases of euphoria and depression. It produces a feeling of depersonalisation or of split personality of a clearly schizophrenic nature. Among the subjective symptoms are an impression of looking at one's self from a distance, of having lost control of one's real self, of having changed and become more or less, unreal and cut off from the rest of the world. Certain subjects experience visual hallucinations, especially if they are in the dark or their eyes are closed. In many cases, lysergic acid diethylamide makes the patient more accessible to psychoanalysis by improving contact and facilitating the recall of memories. It has been used for diagnostic and therapeutic purposes in psychiatry.

5-hydroxytryptamine is known to be present in the brain (Amin, Crawford and Gaddum, 1952). These effects of lysergic acid diethylamide in normal persons may be due to the antagonistic action of the drug on the normal amounts of 5-hydroxytryptamine in the brain. It may be that one of the functions of 5-hydroxytryptamine is to keep one sane.

There/

There are, as yet, no experimental data to support the idea of such a role for 5-hydroxytryptamine. Moreover, lysergic acid diethylamide does not antagonise the effects of 5-hydroxytryptamine on all tissues.

#### Summary

1. A systematic search for a potent and specific antagonist for 5-hydroxytryptamine was carried out. This required the selection of suitable sensitive tissues and the development of quantitative methods for analysing the potencies of these antagonists.

2. 5-hydroxytryptamine causes contractions of the following isolated organs: uterus, duodenum and colon of the rat; the uterus, duodenum and jejunum of the rabbit and the uterus, duodenum, jejunum and ileum of the guinea-pig. It also caused vasoconstriction in the perfused ear of the rabbit.

3. Of these tissues, the rat's uterus, the guinea-/

guinea-pig's ileum, and the rabbit's ear were especially sensitive and the effects of 5-hydroxytryptamine and various possible antagonists were tested using these preparations.

4. With suitable concentrations of mepyramine, piperoxane and atropine it is possible to inhibit the effects of histamine, adrenaline and acetylcholine without altering the responses to 5-hydroxytryptamine. This supports the view that 5-hydroxytryptamine acts on specific receptors which have been called tryptamine receptors.

5. The effects of various 5-hydroxytryptamine antagonists can be explained on the theory that there are two types of tryptamine receptors. One type is present in the smooth muscle of the uterus and ear and is specially inhibited by low concentrations of lysergic acid diethylamide. The second type is present in the nervous tissue in the ileum and is not inhibited by lysergic acid diethylamide.

6. The effect of 5-hydroxytryptamine on the ileum is inhibited by atropine or cocaine in the same way that the effect of nicotine is inhibited. However/

ever, hexamethonium suppresses the response to nicotine but not to 5-hydroxytryptamine. These facts can be explained on the theory that the ganglia in the guinea-pig's intestine contains two types of receptors, one of which is stimulated by nicotine and the other by 5-hydroxytryptamine.

7. The effect of cinobufotenin, the quaternary base corresponding to 5-hydroxytryptamine, is partly inhibited by hexamethonium or by large doses of 5-hydroxytryptamine and completely suppressed by cocaine. It is therefore considered to act on both types of receptors in the ganglia in the guinea-pig's ileum.

8. Ephedrine potentiates markedly the action of 5-hydroxytryptamine on the rabbit's ear, while choline-p-tolyl ether potentiates in low concentrations and inhibits the effects of 5-hydroxytryptamine in higher concentrations. This potentiation of the effect of 5-hydroxytryptamine is presumably due to the inhibition of amine oxidase.

9. A preliminary study with 18 indole compounds showed that the oestrous uterus of a rat was/

was a suitable preparation for the quantitative study of antagonists for 5-hydroxytryptamine.

10. Anti-5-hydroxytryptamine potencies of a large series of compounds were estimated by finding the drug ratio and also, in the case of active compounds, by measuring the dose ratio and the  $PA_2$  values. All these measurements were made at the standard time of 60 minutes.

11. The dose ratio is  $A/A_0$  where  $A_0$  = the concentration of the agonist (HT) which has a given effect (Y) in the absence of the antagonist and A - the concentration of the agonist which has the same effect in the presence of the antagonist in concentrations B and at time t.

12. The drug ratio ( $A/B$ ) is equal to the ratio of the concentration of the agonist to the concentration of the antagonist when the response is 50 per cent of the initial maximum effect.

13. The method of finding the  $PA_2$  was that described by Schild (1947) with slight modification, and gave the most constant results.

14./

14. With most active compounds the effects develop gradually and are irreversible or partly reversible while the effects of less active compounds are complete within a short time and are easily reversible.

15. The compounds tested were derivatives of indole, gramine, tryptamine, carbazole and lysergic acid. Dibenamine, N-ethyl-N-1-naphthyl-methyl-2-bromo ethylamine (SY-28), mepyramine, diphenhydramine and atropine were also investigated.

16. Regarding the relation between chemical structure and pharmacological action, the chemical nature of the side chain of these compounds was found to be important for specificity and potency in their antagonisms to 5-hydroxytryptamine.

17. Lysergic acid diethylamide, dibenamine, dihydroergotamine, dihydroergokryptine and 5-benzyl-oxy gramine in descending order of potencies were the most active antagonists for 5-hydroxytryptamine found.

18. The possible physiological role of 5-hydroxytryptamine is discussed.



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